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ABSTRACT

Upper gastrointestinal bleeding (UGIB) is a medical emergency. There is no precise information of its prevalence and prognosis in patients. The aim of present study was to investigate the prognostic factors of UGIB in patients. In this prospective observational study 75 patients with UGIB referred to Hospital. Demographic and clinical data of them were recorded and analyzed. Mortality rate in first hospitalization was 16% and in one-month follow up was 4%. There was no significant association between age and gender with duration of hospitalization and one-month prognosis ($p>0.05$). Mortality was associated with acute abdomen and orthostatic hypotension I admission time, peptic ulcer in endoscopic evaluation, active bleeding, ICU admission and need to second endoscopy ($p<0.05$). Erosive gastritis, need to emergent surgery and use of NSAIDs significant increase of mortality rate ($p<0.05$). It seems admission time signs and symptoms, hemodynamic and coagulation status, endoscopic results and need to re-endoscopic evaluation are more prognostic factors in patients with UGIB.

KEY WORDS: SHORT TERM PROGNOSIS, UPPER GASTROINTESTINAL, BLEEDING

INTRODUCTION

The UGIB bleeding occurs frequently and is a common cause of hospitalization or inpatient bleeding. Such bleeding results in substantial patient morbidity, mortality and healthcare expense. Ulcer disease is the most common cause of severe UGIB, causing about 40-50% of cases and UGIB is the most common complication of peptic ulcer disease (Kovacs and Jensen, 2008). The initial management of the patient with UGIB should include evaluation of severity of the hemorrhage, patient resuscitation, a brief medical history and physical examination,

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and consideration of possible interventions (Lin et al. 2005). This lack of evidence is reflected in the literature. In databases and in product monographs for corticosteroids, peptic ulcer disease and GI bleeding may or may not be described as possible adverse effects (de Abajo et al. 2013).

GI bleeding, bleeding peptic ulcer and perforation are feared complications of peptic ulcer disease, associated with considerable morbidity and mortality (Lanas et al. 2011). In clinical recommendations, an association between corticosteroid use and peptic ulcer has been described as unlikely, and the value of antiulcer prophylaxis has been questioned due to a low bleeding risk (Martinek et al. 2011). Non-steroidal anti-inflammatory drugs (NSAID) use and Helicobacter pylori infection are the most important risk factors for peptic ulcer disease. Bleeding or perforation is also seen as complications to stress ulcers among patients with critical illness in intensive care units. GI bleeding and perforation are assumed to occur when ulcers erode into underlying vessels (Halliday et al. 2010). It is reported a high late as well as early mortality for upper GI bleeding, with very poor longer term prognosis following bleeding due to malignancies and varices. Aetiologies with the worst prognosis were often associated with high levels of social deprivation (Roberts et al. 2012).

The aetiology of hemorrhage can be broadly considered to be non-variceal or variceal in origin. In the upper gastrointestinal haemorrhage accounts for85% of presentations, the major causative lesion being peptic ulcer disease, followed by erosive diseases such as oesophagitis, gastritis and duodenitis (Jairath & Barkun, 2012). Risk factors for developing upper gastrointestinal hemorrhage include old age, socio-economic disadvantage, co-morbidities such as chronic renal disease, Helicobacter pylori infection and several pharmaceutical agents including NSAIDS, aspirin, cyclo-oxygenase (COX) 2 inhibitors and anticoagulants. The remaining presentations (10–15%) are secondary to variceal hemorrhage in patients with liver cirrhosis (Holcomb et al. 2015).

Coagulopathy exacerbates bleeding and should be corrected with blood products. Massive bleeding mandates emergency endoscopy. Emergency endoscopy is performed as soon as the patient is stabilized after initial resuscitation. In patients with exigent bleeding, endoscopy can be performed during resuscitation (Button et al. 2011). Local studies indicate that as the incidence of upper GI bleeding has increased over time (Hearnshaw et al. 2010). There is also current interest in whether prognosis for emergency disorders varies according to the day of admission, the size of hospital and the distance travelled to hospital although little has been reported about these for upper GI bleeding (Shaheen et al. 2009). So, the main objective of this study was to establish short term prognosis in patients with UGI bleeding in 1 month follow-up period.

MATERIAL AND METHODS

This prospective observational study was done to assess short term prognosis in 75 patients with upper gastrointestinal bleeding referred to the Imam Husein Hospita, (Tehran, Iran) during the 2015-16. The demographic information was collected using check list based on patient's background, Para clinic information, hospitalization and follow-up data. Correlation between sex, gender, age, disease and medication background, gastrointestinal disorder, smoking or alcohol drinking, blood factors (hemoglobin, PT, PTT, INR and platelet) and endoscopic ulceration are determined at the arrival and after 1 month. Data is analyzed by repeated measure one-way analysis of variance (ANOVA) using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). For treatment showing a main effect by ANOVA, means compared by Tukey–Kramer test. $P<0.05$ was considered as significant differences between treatments.

RESULT AND DISCUSSION

As seen in the current study, among 75 patients, 54 male (72%) and 21 women (28%) were include into the study. The mean age of the patients was 61.2 ± 18.6 years old (P=0.4).in this study, 37 (49.3%) had hematoma while 46 (61.3%) had melena, 11 (14.7%) Hematochezia and 27 (36%) with active bleeding were referred to the hospital. In the initial investigation the mean blood pressure and diastolic pressures were 116.1±19.2 and 69.8±23.4 mmHg, respectively. Mean heart rate was 81.2±27.7/min. the clinical results of the patients included into the study is presented in table 1. According to the data, the man HB at the beginning and end of the study were 9.3 ± 2.9 and 9.8 ± 2.01 mg/dl, respectively. The mean PT, PTT and INR were 14.7 ± 8.7, 31.9 ± 25.2 and 1.5 ± 1.4, respectively.

The patient's distribution based on disease background is presented in table 2. According to the data, Hypertension and Cardiovascular disease were the most frequent among them. Also, ulcer was the prominent digestive disease 27 (36%). For family background for gastrointestinal disease, GI cancer 4 (5.3%) was the most report.

Based on the endoscopic observation, clean base and gastritis were the more problem 23 (30.7%) and 22 (29.3%) in the patients.

In this study, 4 patients (5.3%) needed for urgency surgery and 33 (44%) for packed cell. During the study,

Table 1. The clinical results of the patients included into the study

Factor	Minimum	Maximum	Mean ± Sd
Hb (at arrival) (mg/dl)	2.9	16	9.3 ± 2.9
Hb (after treatment) (mg/dl)	6.8	15.7	9.8 ± 2.01
PT	1.3	50	14.7 ± 8.7
PTT	1	120	31.9 ± 25.2
INR	1	9.3	1.5 ± 1.4
Platelet	31500	479000	213200 ± 111800

Table 2. The patients distribution based on disease background

Factor	N (%)	Family background for Gastrointestinal disease	N (%)
Disease background		Ulcer	2 (2.7)
Diabetes	31 (41.3)	Gastrointestinal cancer	4 (5.3)
Hyperlipidemia	17 (22.7)	Intestinal ulcers	2 (2.7)
Hypertension	41 (54.7)	**Medication Background**	
Cardiovascular disease	41 (54.7)	NSAID	20 (26.7)
Cirrhosis	8 (10.7)	SSRI	13 (17.3)
Hepatitis	5 (6.7)	Warfarin	10 (13.3)
Digestive disorders		Steroid	2 (2.7)
Ulcer	27 (36)	**Opioid therapy**	
Intestinal ulcers	8 (10.7)	Alcohol	16 (21.3)
Gastrointestinal cancer	6 (8)	Opiates	31 (41.3)
Gastrointestinal bleeding	8 (10.7)	Smoking	5 (6.7)
		Methadone	1 (1.3)

16 patients needed for hospitalization (21.3%) in the ICU. 6 (8%) of them had bleeding and 11 (14.7%) subsequent endoscopy. Hospitalization period was 5.5±2.5 days and 12 deaths during hospitalization and 3 deaths in 1 month follow-up were reported. After a month follow-up, digestive signs 32 (50.8%), bleeding 32 (50.8%), sever moral 2(3.1%), re-endoscpoy 2(3.1%), re-hospitalization 2(3.1%), and death 3(3.4%) were recorded. No significant difference detected between sex, age, family background, medication, Hematochezia, hematoma and melena before and after the study (P>0.05). No significant difference observed in group aged <60 and >60 years old in mentioned factors (P>0.05).

According to the results, a significant correlation observed between blood pressure and bad moral (P<0.001), GI bleeding (P=0.018), alcohol (P=0.04), Opium administration (P=0.000), NSAIDs (P=0.036), ulcer incidence in primary endoscopy (P=0.003), Gastritis (P=0.008), emergency surgery (P=0.000), ICU care (P=0.000), re-bleeding (P=0.010) and need for subsequent endoscopy (P=0.000). A correlation exist between opium administration in 1 month follow-up and endoscopic ulcer (P=0.07). A significant correlation reported between death and peritonitis (P=0.046), hypotension (P=0.053), gastric ulcer in endoscopy (P=0.04), bleeding (P=0.023), ICU care (P=008) and no differences found in re-endoscopy (P=027), NSAIDs (P=056), gastritis (P=073) and emergency surgery (P=053).

According to the results, mortality rate in first hospitalization was 16% and in one-month follow up was 4%. There was no significant association between age and gender with duration of hospitalization and one-month prognosis. Mortality was associated with acute abdomen and orthostatic hypotension I admission time, peptic ulcer in endoscopic evaluation, active bleeding, ICU admission and need to second endoscopy. Erosive gastritis, need to emergent surgery and use of $NSAID_s$

Table 3. The endoscopic observation in the patients

Endoscopic results	N (%)
Pigmented ulcer Pigmented ulcer	2 (5.3)
Clean base	23 (30.7)
Gastritis	22 (29.3)
Tearing	2 (2.7)
Gastric *varices*	7 (9.3)
Visiblessle ulcer	8 (10.7)

significant increase of mortality rate. It seems admission time signs and symptoms, hemodynamic and coagulation status, endoscopic results and need to re-endoscopic evaluation are more prognostic factors in patients with UGIB.

In a study, Lanas et al. (2011) on 2660 patients (64.7% men; mean age 67.7 years) significant differences reported on across countries in bleeding continuation / re-bleeding (range: 9–15.8%) or death (2.5–8%) at 30 days were explained by clinical factors (number of comorbidities, age > 65 years, history of bleeding ulcers, in-hospital bleeding, type of lesion or type of concomitant medication). Other factors (country, size of hospital, profile of team managing the event, endoscopic and/or pharmacological therapy received) were not able to affect these outcomes (Loperfido et al. 2009). Risk factors that have been previously identified to be predictive of bleeding continuation and re-bleeding include presence of comorbidities outcomes (Loperfido et al. 2009) endoscopy-observed high-risk stigmata of bleeding; worse health status at admission; bleeding from a peptic ulcer (Viviane and Alan, 2008) a finding of bright blood during rectal examination and in nasogastric tube aspirate; smoking; failure to use PPIs postendoscopy; postendoscopy use of intravenous or low molecular-weight heparins and low endoscopist experience (Travis et al. 2008).

A number of these previously identified predictive factors were confirmed in UGIB (i.e. presence of comorbidities, bleeding from a duodenal ulcer), and a number of new predictors of bleeding continuation/ re-bleeding were characterized: older age (>65 years), presentation with haematemesis and a history of UGIB at baseline (Button et al. 2011). Previously characterized predictors of mortality include older age; presence of, and increasing number of, comorbidities; continued bleeding and / or re-bleeding and a finding of bright blood in the nasogastric tube aspirate (Marmo et al. 2010). The predictive validity of older age and the presence of comorbidities were confirmed in UGIB; in fact, the presence of comorbidities was by far the strongest predictor of mortality in this patient population. Other factors identified to be significantly predictive of mortality in this study were presentation with clinical symptoms of acute upper GI bleeding and alcohol abuse (Shaheen et al. 2009).

The overall rate of deaths due to GI complications and the rate of deaths associated with NSAID/aspirin use reported are lower than some frequently quoted estimates from previous studies, despite the fact that our figures include both upper and lower GI complications and also refer to low-dose aspirin use (Hawkey and Langman, 2003).

There are a number of reasons that may account for some of the discrepancies observed in the studies: variation in prescribing practice by country; differences in the extent of NSAID use and in the co-prescription of gastroprotective drugs; decreasing GI complication rates; and differences in study methodologies. Our data would imply a lower NSAID consumption in Spain compared with other countries. However, the annual NSAID prescription rates in Spain are relatively high (35.4 million) (Van Leerdam et al. 2003) and are proportionally greater than rates reported in the United Kingdom and 50% of those reported in the United States (70 million), despite Spain's smaller population. In addition, the rate of NSAID use among adults in Spain (20.6%) is similar than that determined in the United States (Estudio, 2000). Upper GI bleeding is one of the most important emergency disorders with high rates of mortality during the acute phase. Longer term increased risk of mortality is partly due to very poor prognosis for malignancy and variceal etiologies; although it also reflects an impact of high levels of social deprivation and chronic co-morbid disease among people with upper GI bleeding (Roberts et al. 2012).

Survival over the three years was substantially poorer than in the general population for most etiologies of bleeding, with the possible exception of 'complications of analgesics, antipyretics and anti-inflammatory drugs' and duodenal ulcers, which were both less prevalent among deprived quintiles than most of the other etiologies. Relative survival was worse for duodenal ulcer than for gastric ulcer in the first few months after admission, but it was better than for gastric ulcer bleeds in the longer term. This finding is consistent with a large single-center study of surgery for peptic ulcer which found increased longer term mortality for gastric ulcer but not for duodenal ulcer (Stae¨l von Holstein et al. 1997). In conclusion it seems admission time signs and symptoms, hemodynamic and coagulation status, endoscopic results and need to re-endoscopic evaluation are more prognostic factors in patients with UGIB.

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Resilience and basic psychological needs among Palestinian school students

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ABSTRACT

The purpose of this study is to explore the relationship between Basic Psychological Needs including Autonomy, Competence and Relatedness (Deci and Ryan, 2000) and factors predicting resilience (American Psychological, 2010) among Palestinian school students who are living under adversity in the West Bank. The participants were 537 students 13 and 14 years old (45% male and 55% female) representing both urban and rural areas of the northern West Bank. All participants completed the CYRM-28 Psychological Resilience Questionnaire (Liebenberg et al., 2012) and The Basic Psychological Needs Scale-General Version (Ilardi et al., 1993). Structural Equation Modeling (SEM) analysis results showed that the BPN model adequately explained variable variance (MOD FIT/CFI = 0.998) and that satisfying Basic Psychological Needs had positive and significant effect on resilience factors of Caregiving (Physical and Psychological Caregiving), Individual (Personal Skills, Peer Support, and Social Skills), and Context (Spiritual, Education and Cultural Context). The role of (BPN) was significant ($\beta = 0.297$, $p < 0.001$), ($\beta = 0.409$, $p < 0.001$), and ($\beta = 0.241$, $p < 0.001$) respectively Caregiving, Individual, and Context, factors were high (0.711, 0.706, and 0.80) respectively, which in turn indicated that (BPN) plays strong role in explaining the variance of Caregiving, Individual, and Context factors. Based on these findings, (SDT) can predict Resilience factors in case of satisfying (BPN). Findings of the study support that educational and family practices focusing on satisfying psychological needs are related to childhood resilience in the face of adversity.

KEY WORDS: RESILIENCE, SELF-DETERMINATION (SDT), AND BASIC PSYCHOLOGICAL NEEDS (BPN)

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INTRODUCTION

Palestine is a nation in a unique geo-political situation where violence imposed by armed forces and/or military violence as well as with restriction of movement through checkpoints, closures and curfews, and acts of individual and communal threat and humiliation occur regularly. Traumatic events such as shootings, bombings, destruction of houses, physical assaults and deaths occur in some areas on a daily basis Rytter et al., 2006, Abdeen et al., 2008, Soares et al., 2007, Qouta et al., 2008a, Thabet et al., 2016, Thabet, 2015, Mousa Thabet and Vostanis, 2017, Al-Sheikh and Thabet, 2017).

Numerous studies have noted distinct psychological and behavioral impacts of traumatic experiences during situations of political unrest on youth, related to psychological health, well-being, and long term outcomes, including increased risk of suffering from mental health problems, such as; PTSD, insomnia, depression, low feeling of self-efficacy and self-esteem, anxiety and depressive symptoms, cognitive distortions, and behavioral disturbances (Worden, 1996, Saigh et al., 1995, Chimienti et al., 1989, Foa et al., 1999, Baker, 1990, Stubbs and Soroya, 1996, Garbarino and Kostelny, 1993, Moro et al., 1998, Clarke et al., 1993).

It has also been demonstrated that youth in protracted conflict zones exhibit increased difficulties in social relationships, fear of the dark, phobias, bedwetting, social withdrawal, negative social-interaction, aggressive behavior, insecure attachment, forgetfulness, somatic disorders and psychosocial behavioral problems, fear, anger, sadness, humiliation, guilt, nightmares and emotional disregulation (Giaconia et al., 1995, Punamäki, 1997, Foa et al., 1999, Vila et al., 1999, Qouta et al., 2008b), as well as academic challenges such as low grades, concentration difficulties, and truancy from school (Qouta and El-Sarraj, 2004, Kanninen et al., 2003, Thabet and Vostanis, 2000, Altawil, 2008). These indicators reveal how difficult it is for children residing in high conflict zones, such as Palestine, to have a normal developmental trajectory and the high risk for negative lifespan risks related to childhood trauma. Given the high number or young people exposed to traumatic events in Palestine, it is necessary for individual, community, and national well-being and progress to identify protective factors to reduce the potentially negative impacts of this currently inevitable exposure to violence (Hobfoll et al., 2011, Nguyen-Gillham et al., 2008, Thabet and Thabet, 2015a, Thabet and Thabet, 2015b).

Deci and Ryan (2000) Proposes that the relationship between the social contextual environment and people's well-being is critical for positive human development in Self-Determination Theory (SDT). Self-Determination Theory is based on the tenant that the fulfillment of three Basic Psychological Needs (BPN) including autonomy, competence and relatedness, is essential for positive functioning and when these basic psychological needs are fulfilled, optimal psychological well-being should occur (Gunnell et al., 2013). Ryan and Deci (2000) argue that satisfaction of the (BPN) for autonomy, competence, and relatedness improves well-being, and strengthens inner resources related to resilience, whereas frustration in these three areas increased vulnerability for defense mechanisms and psychopathology (Weinstein and Ryan, 2011, Vansteenkiste and Ryan, 2013). Weinstein and Ryan (2011), proposed that psychological need satisfaction acts as a buffer in times of stress, reducing both initial appraisals of stress and encouraging adaptive coping after stress-related events occur.

The model of motivational resilience used in this study is based on Self-Determination Theory (SDT) (Deci and Ryan, 1985), and organized around the assumption that all individuals aim to satisfy the basic psychological needs of competence, relatedness, and autonomy. According to this perspective, humans inherently seek to explore opportunities to satisfy these needs. Individuals feel energized and joyful during interactions in which their needs are satisfied, and frustrated when they are thwarted. Based on their history of experiences in particular situations, people construct views of themselves and the world in relation to these needs. Over time, these expectations come to shape their participation in their environment (Skinner et al., 2014).

The construct of resilience has been broadened from those experiencing severe environmental disruption to include the general population with everyday stressors and difficulties (Timmerman, 2014, Martin and Marsh, 2008). Resilience is intricately related to behavioral autonomy, self-realization, self-regulation, and psychological empowerment (Weston and Parkin, 2010). Resilient individuals exhibit behavioral autonomy in taking responsibility for their actions. Individuals who are resilient are most likely to possess high levels of self-realization and self-efficacy (Timmerman, 2014). Moreover, resilient individuals do not shy away from challenging tasks but exert even more effort, use more effective strategies, and approach difficult tasks with persistence. Resilient individuals self-regulate by planning for and setting goals and consequently monitoring their progress toward these goals. People who exhibit self-realization are aware of their strengths and abilities, reflect upon their past successes with challenging events, develop self-efficacious beliefs in their abilities, and demonstrate greater capacities for responding to future events with resilience (Timmerman, 2014). In addition, research shows a strong association between psychological empowerment and resilience (Pines et al., 2012).

Numerous scholars have noted the bond between resilience and self-determination. Spreitzer (1995) stated that, persons who are empowered or self-determined demonstrate greater resilience. Empowered individuals display resilience, self-determination, power, control, ability, competence, self-efficacy, autonomy, knowledge, and development (Uner and Turan, 2010).

Based on the previous research, the satisfaction of basic psychological needs, facilitated by supportive social contexts, appears to foster both a sense of wellness and leads to the building of inner resources that underlie subsequently demonstrated resilience.

The current study was designed to explore the association between factors of resilience factors and the satisfaction of Basic Psychological Needs among Palestinian youth in West-Bank Directorates by using structural equation modeling including:

- The association between (BPN) and Resilience

MATERIAL AND METHODS

PARTICIPANTS AND PROCEDURE

The sample consisted of 537 Palestinian public school student's 13 and 14 years old living in the West Bank (OPT Occupied Palestinian Territories). They were 55% girls and 45% boys. About two thirds (64%) were from rural areas and (36%) from urban areas. For the study, 25 schools were randomly selected as representative of schools in the North directorate of the West Bank. At each school 10 students from 8th grade and 10 students from 9th grade, were randomly selected. The High Ministry of Education provided the permission to access the public schools, and then researcher informed the pupils, their parents, and headmaster about the purpose of study, obtaining their verbal consent for participation.

MEASURES

The Child and Youth Resilience Measure [CYRM]

(Liebenberg et al., 2012) is a comprehensive instrument composed of three sub-scales, which reflect the major categories of resilience. The first sub-scale is "Individual Factors" that included personal skills (5 items), peer support (2 items) and social skills (4 items). The second subscale is labeled "Family Support", as reflected in physical/material support (2 items) as well as psychological care giving (5 items). The third sub- scale is "Contextual Components" which are environmental characteristics that facilitate a sense of belonging in youth, including spirituality (3 items), culture (5 items), and education (2 items). All responses were measured on a Likert Scale from 1 to 5 (1 = "never" and 5 = "always"), (Liebenberg et al., 2012). The Cronbach alpha coefficients were calculated for each dimension in the CYRM-28, (individual factors, family support, and contextual components) 0.80, 0.78 and 0.84 respectively. Cronbach's alpha for the total scale (28 items) was 0.92.

The (BPN) Scale-General Version contains 21 items and is adapted from the (BPN) -work version (Ilardi et al., 1993). Responses for all items were indicated on a Likert Scale from 1 (not true at all) to 7 (definitely true) (Ryan and Deci, 2000). The instrument includes three sub-scale scores, measuring the degree to which the person experiences satisfaction of each of the three needs.

DATA ANALYSIS

Structural equation modeling (SEM) using Analysis of Moment Structure (AMOS) (SPSS Version 21) was used to analyze the data. Confirmatory Factor Analysis [CFA] and Path Analysis were used to test psychological resilience related to the factors of individual characteristics, caregiving, contextual components and the Basic Psychological Needs related to autonomy, competence, and relatedness. This study anticipated a positive path from (BPN) to factors of Psychological Resilience (Clauss-Ehlers, 2008, Pines et al., 2012, Ruban et al., 2003, Deci and Ryan, 2000, Skinner et al., 2014, Spreitzer, 1995, Timmerman, 2014, Uner and Turan, 2010, Wehmeyer, 1996, Weston and Parkin, 2010, Tuckman, 2003).

Figure 1, shows the proposed model:

H^A: There is positive path from (BPN) to factors indicating Psychological Resilience including individual characteristics, caregiving, and context. (See Figure1).

Table 1. Demographic Characteristics for the Participants

Demographic variables (n = 537)		Frequencies	(Valid Percentage n = 537)
Gender	Males	242	45
	Females	295	55
Age	13	268	50
	14	269	50
Place of residence	City	196	34
	Village	341	66

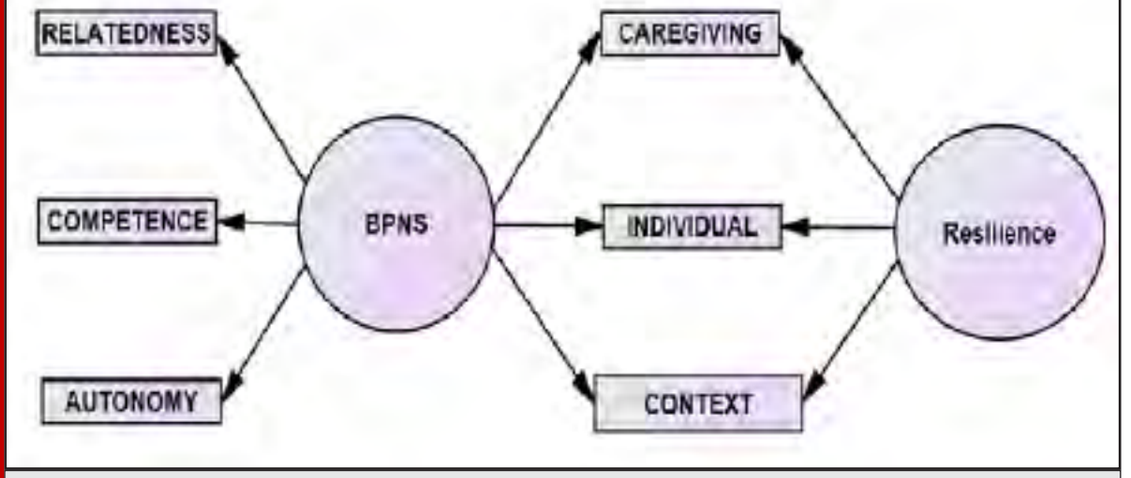

FIGURE 1. Theoretical model psychological resilience factors and its relationship with satisfaction of BPNS among the Palestinian basic school students in West-Bank.

RESULTS AND DISCUSSION

DESCRIPTION OF (BPN) AND RESILIENCE

The researchers computed means and SD for (BPN) and its domains (Autonomy, Competence, and Relatedness) and for Resilience and its subscales (Individual Characteristics, Caregiving, and Context), (see table 2).

CONFIRMATORY FACTOR ANALYSIS OF THE COMPONENTS OF (BPN) AND RESILIENCE

The results indicate that factor loadings were higher than .50, ranging from 0.66 to 0.7 for the (BPN), and higher for the Resilience components from 0.73 to 0.86, At the construct level, the results demonstrate that the reliabilities of all of the constructs ranged from 0.70 to 0.86 (higher than the recommended cut-off of 0.70 for this measure).

THE ASSOCIATION BETWEEN (BPN) AND RESILIENCE COMPONENTS

The present study utilizes SEM to test the hypothesized model regarding the relationship between the component variables of (BPN) and Resilience factors including: Individual Characteristics, Caregiving, and Context. The model predicts a positive path from (BPN) to Individual Characteristics, Caregiving, and Context, (the pre-identified components of resilience). Table 4 presents the data on statistical fit for the hypothesized model, using standardized paths coefficients (Beta), and the estimate of variance explained (R^2).

The χ^2 value was 8.489 (d.f. = 6, p = 0.204). Therefore, the relative χ^2 was (CMIN/df = 1.415). The RMSEA estimate of 0.028 provided support for the general model. Bentler's CFI was 0.998 indicating that the proposed model fit the data according to this index.

Table 2. Means and standard deviations of total resilience and resilience factors of children (N = 537)

Descriptive Statistics			
Constructs	**Mean**	**Std. Deviation**	**N**
BPNS	5.1485	.68308	537
Autonomy	4.9327	.90893	537
Competence	5.0636	.83385	537
Relatedness	5.4493	.80478	537
RESILIENCE	3.7956	.67234	537
Individual Factors	3.4708	.62142	537
Caregiving Factors	4.0093	.81419	537
Context Factors	3.9067	.79080	537

Table 3. Standardized Path Coefficient and P value

Parameter Description	**Standardized Path Coefficient (β)**	**P Value**
Relatedness from BPNS	0.666**	0.000
Competence from BPNS	0.640*	0.000
Autonomy from BPNS	0.749**	0.000
Context from Resilience	0.861**	0.000
Individual from Resilience	0.734**	0.000
Caregiving from Resilience	0.789**	0.000

Table 4. Model Fit Indices and Recommended Values for SEM Analysis (Kline, 2005)

Model Fit Index	Model Fit summery	Recommended Values
CMIN (Chi-square p value)	0.204	> .05
CMIN /df	1.145	≤ 3
CFI	0.998	≥ .90
GFI	0.995	≥ .90
AGFI	0.982	≥ .90
NFI	0.994	≥ .90
RMSEA	0.028	≤ .05

The goodness of fit index (GFI), adjusted GFI (AGFI), and Normed fit index (NFI) for the measure were 0.995, 0.982, and 0.994 respectively; demonstrating general fit. Path coefficients from (BPN) to INDIVIDUAL, CAREGIVING, and CONTEXT as well as total Resilience were significant (see Table 4 and 5).

As shown in table 4 and 5, the standardized path coefficient of caregiving factors from (BPN) was significant ($\beta = 0.297$, $p < 0.001$), R^2 (0.711), indicating that (BPN) plays a strong role in explaining the variance of caregiving.

Perditions from Individual Characteristics to (BPN) it was significant ($\beta = 0.409$, $p < 0.001$), R^2 for individual factors was high (0.706), which in turn indicated that (BPN) plays strong role in explaining the variance of individual factors; (Individual Personal Skills, Individual Peer Support, and Individual Social Skills).

The variability of Context factors explained by (BPN) it was significant ($\beta = 0.241$, $p < 0.001$). R^2 (0.80), which in turn indicated that (BPN) plays strong role in explaining the variance of context factors.

To conclude, figure 2 shows SEM for Resilience factors and their relationship with satisfaction of (BPN).

As shown in the pervious model and tables 4 and 5, the results revealed that basic psychological needs were significantly and positively associated with the factors of resilience; (individual, caregiving, and context) among the participants.

DISCUSSION

The study aimed to explore whether the satisfaction of basic psychological needs (BPN) affected resilience factors, in a sample of middle-school students living under situations of geo-political adversity, in Palestine.

Results of the current investigation revealed that the general level of Resilience and (BPN) measures in the sample was high. Additionally, the results revealed that there is significant relationship between (BPN) and the selected factors of Resilience (individual characteristics caregiving, and context), especially between BPN and Individual Characteristics.

The results demonstrate that satisfying basic psychological needs of youth in high conflict environments, has positive effect on resilience and well-being. By reviewing these results it can be supposed that satisfying basic psychological needs positively predicts resilience which is consistent with the explanation offered under self-determination theory (Baard et al., 2004) in which they propose that satisfying basic psychological needs, has a positive effect on resilience and well-being.

Additionally the results of this study are consistent with previous findings that explored (Baard et al., 2004, Deci and Ryan, 2000, Kaydkhorde, 2014), relationships between the children and their families and environmental context as supporting and contributing to satisfaction of basic needs, leading to the facilitation of mental adaptation, resilience and well-being.

Meeting the Basic Psychological Needs of autonomy, competence and relatedness provide essential conditions for positive development and growth, consistency and well-being, (Deci et al., 2001), and may determine a large variance in behavioral functioning (Deci and Ryan, 2017, Sheldon et al., 1996) (Deci and Ryan, 2017).

Table 5. Model Fit Indices, Recommended Values for SEM Analysis, and the Observed Values for the Proposed Model

Model Fit Index	Observed Values	Parameter Description	Standardized Path Coefficient (β)	P Value	R2
Chi-square value	8.489	Relatedness from BPNS	0.666**	0.000	CAREGIVING = 0.711
d.f.	6	Competence from BPNS	0.640*	0.000	INDIVIDUAL = 0.706
CMIN (p value)	0.204	Autonomy from BPNS	0.749**	0.000	CONTEXT= 0.800
CMIN /df	1.145	Context from Resilience	0.861**	0.000	AUTONOMY = 0.561
CFI	0.998	Individual from Resilience	0.734**	0.000	COMPETENCE = 0.409
GFI	0.995	Caregiving from Resilience	0.789**	0.000	RELATEDNESS = 0.443
AGFI	0.982	Caregiving from BPNS	0.297**	0.000	
NFI	0.994	Individual from BPNS	0.409**	0.000	
RMSEA	0.028	Context from BPNS	0.241**	0.000	

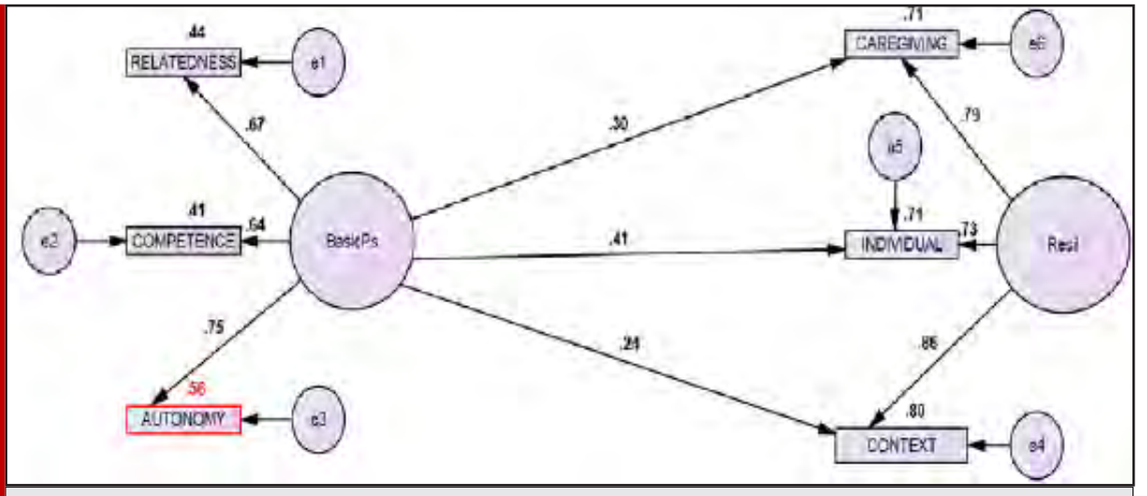

FIGURE 2. SEM for psychological resilience factors and its relationship with satisfaction of BPNS among the Palestinian basic school students in West-Bank.

In conclusion, Self-Determination Theory (SDT) is organized around the satisfaction of three basic psychological needs (BPN) including autonomy, competence, and relatedness. Students' histories of experiences with family, school, and the community; including their interactions with parents, teachers, and peers who support or undermine their needs, cumulatively shape their academic identities, or their personal convictions about whether they truly belong (relatedness), have what it takes to succeed (competence), and genuinely endorse the goals and values of schooling (autonomy) Cleary define their resilience, especially in adversive environments. These self-system processes, along with the nature of the academic work students are given (i.e., whether it is authentic, relevant, purposeful, and important) are the proximal predictors of students' motivational resilience (or vulnerability), including their engagement, coping, and re-engagement (Skinner et al., 2014).

In general, an individual with the skills and characteristics of resilience paired with the traits of self-determined behavior best prepares one to adapt to new environments and increases the utilization of personal responsibility to address pressures they encounter in the environment (Timmerman, 2014). Individuals who capably demonstrate self-determined behaviors and resilience are better able to consistently work toward achieving challenging goals without losing interest or lessening their effort despite hitting a plateau or experiencing outright failure on their first attempt (Timmerman, 2014).

In order to improve resilience among Palestinian children, individual skills factors (personal skills, peer support, and social skills), context factors (spiritually, education and culture) and caregiving factors (physical and psychological) fostering and building suitable educational and academic interventions may be key based on self-determination theory and the current findings. Collective interventions, either at community level or school level may be the best way to effectively address these issues for Palestinean youth and the full range of the population (Rabaia et al., 2010, Pieters, 2016, Hoge et al., 2007).

LIMITATIONS

Despite demonstrating (BPN) effects, it is uncertain that the directionality of the effects is in accordance with the model. To further examine these hypotheses and to provide conclusions with respect to the direction of the effect, it is recommended that future research include longitudinal data and school-change interventions.

Further limitations of the current investigation is the self-report nature of the instruments utilized, it was not possible to know exactly how the students interpreted the constructs and whether they viewed the constructs in ways that the researcher intended. While this is a limitation of all survey self-report research of this nature, it should be noted that the confirmatory factor analysis did provide some support that the students were responding as expected. Future research could overcome this limitation by including a mixed methods approach.

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Effect of heptane food simulating liquid on surface microhardness of 4 composites (Filtek Z250, Aelite, Filtek Z350 and Clearfil ST)

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ABSTRACT

Resin based composites are became more and more popular in restorative dentistry, particularly because of their esthetic aspects. Decreasing the microhardness of dental restorative composites after curing in oral environment can influence their clinical durability. The aim of the current study was to determine effect of food simulating liquids 50% heptane on surface microhardness of Z250 microhybrid, Aelite nanofilled Z350 and Clearfil nanohybrid composites. 20 specimens of each composite were prepared in a prefabricated mold with 5 diameter and 2 mm depth. All the specimens composite were stored in distilled water, immediately after curing for 24 hours as the control group. Then the specimens were taken out of the solution and washed, dried and then surface microhardness of specimens was evaluated by the microhardness device based on Vickers. These specimens were divided into two groups randomly;

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each of them was immersed in one of the following solutions distilled water, 50% heptane for 7 days at 37 °C. After one week conditioning period microhardness testing was carried out. The data were analyzed by 2way ANOVA and Tucky HSD test. According to the results, there were significant differences on the initial microhardness of all composites in water ($p<0.05$). Microhardness of the Z250 was higher than the other groups in water and heptane ($p<0.05$). A significant decrease observed on the secondary microhardness of the Aelite and Clearfil composites in heptane compared to the first time ($p<0.05$). The Clearfil had higher decrease on microhardness in water and heptane compared to the other composites ($p<0.05$). The microhardness of composite resin materials used in this study influenced after immersion in Heptane food simulation solution and distilled water. The effect of heptane on change in surface microhardness is material dependent.

KEY WORDS: COMPOSITE,NANOHYBRID, MICROHYBRID,NANOFILL, HEPTANE, FOOD SIMULATING

INTRODUCTION

The administration of resin-based restorative materials in dentistry has increased recently because of their good aesthetic appearance, improvements in formulations, ease of handling, and ability to establish a bond to dental hard tissues. The mechanical property of the dental composites depends on the filler particles and particle size. Recent advancements on the organic matrix and inorganic fillers have led to the development of new materials with reduced particle size and increased filler loading which improved mechanical properties and aesthetics on the current composite resin materials.Restorative materials are required to have long-term continuousness while the oral cavity is a complex aqueous environment and restorative material contacts with saliva, (Catelan et al. 2010, Hengtrakool et al. 2011, Erdemir et al. 2013 George and Kavyashree 2017).

Also, low pH due to acidic foods and drinks may influence the mechanical and physical characteristics of the materials (Miranda et al. 2011). Physical characteristics of restorative materials are an important concern when determining suitable restorative materials because they strongly influence the clinical longevity of restorations (Seifert et al. 2011). In clinical environment, microhardness of materials decrease might contribute to its deterioration. Under in vivo conditions, composite resin materials may be exposed either discontinuously or continually to chemical agents found in saliva, food and beverages (Topcu et al. 2010). In the short- or long-term, these conditions have adverse effect on its physical and chemical structure (Valinoti et al. 2008). The material's microhardness is one of the most important properties, which correlates with resistance to intra-oral softening, compressive strength and degree of conversion (Voltarelli et al. 2010). A low surface microhardness value is largely related to inadequate wear resistance and proclivity to scratching, which can compromise fatigue strength and lead to failure of the restoration (Erdemir et al. 2013). So, the aim of the current study was to determine effect of food simulating liquids 50% heptane on surface microhardness of Z250 microhybrid, Aelite nanofilled Z350 and Clearfil nanohybrid composites

MATERIAL AND METHODS

In this experimental in vitro study 4 composite types were used (n=10). The composites allocated in stainless steel (5mm diamater×2mm thickness). A smooth plate put on the composite and the produced collected at 40 s by SDS Kerr (1000mW/cm^2) and polymerized (2×2) and polished using aluminum oxide (3M ESPE) by spraying the water. Then samples stored in distilled water 37°C for 24 h. Then microhardness of the samples determined using Intender (6100 Vickers, USA).

COMPOSITES

The information of the composites used in the study was Filtek z250 Micro hybrid (filler weight 82%, filler volume 60%) Zirconia silica (0.6µm) Bis-EMA, UDMA Bis-GMA. The Filtek Z350 was Nanofilled (filler weight 78.5%, filler volume 59.5%) Zro2/sio2 nanocluster, Sio2 nanofiller (5-20nm) Bis-GMA Bis-EMA UDMA TEG-DMA. The Aelite was Nanofilled (filler weight 73%, filler volume 54%) Glass frit Amouphous silica (0.04-5µm) Exhoxylated Bisphenol A Dimethacrylate TEGDMA. The ClearfilMajesty ES-2 was Nanohybrid (filler weight 93%, filler volume 81%) Silanatedbarium glass filler Pre-polymerized organic filler (0.04-1µm) hydrophobic aromatic dimethacyilate TEG-DMA Bis-GMA. The 50 gr force for 15 s is done using Intender on 3 points in each sample. Then the microhardness of the samples determined. The 10 samples allocated into the heptane and 10 in distilled water for 7 days. After one week conditioning period microhardness testing was carried out.

STATISTICAL ANALYSIS

The data were analyzed by 2way ANOVA and Tucky HSD test using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). $P< 0.05$ was considered as significant differences between treatments.

RESULTS AND DISCUSSION

According to the results, there were significant differences on the initial microhardness of all composites in water ($p < 0.05$). Microhardness of the Z250 was higher than the other groups in water and heptane ($p < 0.05$). No significant difference observed on primary microhardness of Aelite and Clearfi l ($p > 0.05$). A significant decrease observed on the secondary microhardness of the Aelite and Clearfi l composites in heptane compared to the first time ($p < 0.05$). The microhardness of Clearfi l significantly decreased compard to the other composites in water and heptane conditions.

As seen in table 2, a significant differences observed between primary and secondary microhardness of the Z350 (65.30±6.19 and 75.84±4.25), 75.84±4.25 (50.88±7.47 and 39.87±5.07), Z250 (73.69±3.69 and 85.22±9.33) and Clearfil (43.66±4.99 and 35.47±4.61).

As seen in table 3, significant difference was observed on microhardness of Aelite (0.006) and Clearfil (0.0001) stored in heptane.

The primary and secondary microhardness of materials is presented in table 4.

Table 1. The microhardness of the different composited composite stored in distilled water or heptane

Composite	Food suspension	Primary microhardness	Secondary microhardness
Z350	distilled water	65.3000	75.8450
	Heptane	63.8390	67.3380
Aelite	distilled water	73.6970	85.2210
	Heptane	77.3370	82.7360
Z250	distilled water	50.8810	39.8760
	Heptane	50.8720	39.8550
Clearfil	distilled water	43.6690	35.4780
	Heptane	43.4300	33.5460

Table 2. the primary and secondary microhardness of composite stored in distilled water

Composite	Primary distilled water	Secondary distilled water	P value
Z350	65.30±6.19	75.84±4.25	0.0001
75.84±4.25	50.88±7.47	39.87±5.07	0.015
Z250	73.69±3.69	85.22±9.33	0.0001
Clearfil	43.66±4.99	35.47±4.61	0.013

Table 3. the primary and secondary microhardness of composite stored in heptane

Composite	Primary heptane	Secondary heptane	P value
Z350	63.83±3.55	67.33±5.95	0.226
Aelite	50.87±6.41	39.85±6.90	0.006
Z250	77.33±6.27	82.73±3.68	0.064
Clearfil	43.43±4.46	33.54±2.62	0.0001

Table 4. the primary and secondary microhardness of materials

Compared materials		t-Test	P value
Z350 & Distilled water (primary & secondary)	-10.54±1.33	-7.906	0.001
Z350 & Heptane (primary & secondary)	-11.52±6.86	-5.312	0.226
Aelite & Distilled water (primary & secondary)	11.00±3.67	2.991	0.015
Aelite & Heptane (primary & secondary)	11.01±3.083	3.573	0.006
Z250 & Distilled water (primary & secondary)	-9.09±8.90	-3.231	0.001
Z250 & Heptane (primary & secondary)	-9.55±10.71	-2.820	0.064
Clearfil & Distilled water (primary & secondary)	8.19±2.66	3.072	0.013
Clearfil &tHeptane (primary & secondary)	9.88±1.35	7.280	0.001

DISCUSSION

During consumption of food or drink contacts teeth or restoration surfaces for only a short time before it is washed away by saliva. Usually contact of teeth with acidic food or drink for a prolonged period of time and the situation did not account for the role of saliva (Erdemir et al. 2013). As observed in the current study, surface microhardness of Z250 was higher than the other groups. After 24 hours distilled water had significant effect on all the specimens. After 7days distilled water had significant effect on all groups however, Heptane had significant effect on Aelite and Clearfil specimens. According to analyses after both 24 hours and 7 days Z250 and Z30 specimens showed increase in microhardness while Aelite and Clearfil showed significant decrease in microhardness. Clearfil presented the lowest microhardness values. Distilled water was selected instead of artificial saliva to simulate the aching effect of saliva because the artificial saliva storage medium is not considered to be a more clinically relevant environment (Erdemir et al. 2013).

The surface microhardness index of all restorative materials after a week of storage in distilled water was higher than the baseline surface microhardness val-

ues. This could possibly be explained by the amplified monomer conversion and additional post-curing cross-linking reactions in the resin phase over the time. Compoglass F, Filtek Z250, Filtek Supreme and Premise specimens stored in distilled water had lower surface microhardness reductions compared to the specimens immersed in sports and energy drinks (Erdemir et al. 2013). In a study using Meliodent, FuturaGen and hard GC reline.

Rajaee et al. (2014) reported heptane conditioning decreased the flexural strength of Meliodent and FuturaGen and microhardness of FuturaGen. Ethanol solution had the most adverse effect on the microhardness and flexural strength of the tested resin materials (Rajaee et al. 2014). Takahashi et al. (1998) reported that water immersion had different effects on the flexural strength and microhardness of different denture base and reline resin materials. They concluded that the results could be due to the fact that the intrinsic strength of the resin and the amount of water sorption in the system influences the mechanical strength of water absorbed acrylic resins. It is reported two days of immersion in the water lead to a reduction in the microhardness of the resin samples. As mentioned, water absorption and continuation of the acrylic polymerization process is time-dependent and diffusion-controlled Azevedo et al. (2005). Organic solutions may damage the resin matrix (heptane and aqueous ethanol solution). On the other hand, water and citric acids can damage organic fillers. Therefore organic solutions could decrease flexural strength and microhardness of dental resins (Yesilyurt et al. 2009).

In a study, Yanikoğlu et al. (2009) determined the surface microhardness of filled (Estelite), nanofil (Ælite), unfilled (Valux Plus), hybrid (Tetric ceram) and Ormocer-based (Admira) composite resins in tea, coffee, Turkish coffee, mouthwash, cola, and distilled water. Based on their report the microhardness values of composite materials were statistically different in different immersion solutions. The acidity may change the polymeric matrixes of composite resin affecting dimethacrylate monomer present in their compositions (Al-Samadani, 2013). A previous study suggested that, by lowering the solutions' pH, there is production of methacrylic acid that results in the sorption and hygroscopic expansion as a consequence of enzymatic hydrolysis and biodegradation (Sripetchdanond and Leevailoj, 2014). It was observed that sodium fluoride containing mouth rinses also reduce the surface microhardness (Sripetchdanond and Leevailoj, 2014).

In a recent study, George et al (2017) on effect of four mouth rinses on microhardness of resin composite (Filtek™ P60) material (3M ESPE St. Paul, MN, USA) reported all the mouth rinses showed reduction in surface microhardness of the esthetic restorative material. Yesilyurt et al. (2009) reported microhardness of silorane-based composite was not influenced by ethanol significantly, which could be due to the hydrophobicity of the resin matrix. Except for Bis-EMA, all other molecules (Bis-GMA, UDMA, and TEG-DMA) have hydroxyl groups, which promote water sorption. As for silorane-based composite, it has 3,4-epoxycyclohexyl-cyclopolymethylsiloxane. In conclusion, the microhardness of composite resin materials used in this study influenced by food simulation solutions. The effect of heptane on change in surface microhardness is material dependent.

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Antibacterial and antioxidant activity of leaf organic extracts of local cultivars of *Murraya koenigii* (L.) Spreng from Tamilnadu

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ABSTRACT

The aim of the present study was to evaluate the antibacterial activity against five bacterial pathogens and anti-oxidant activity of three organic extracts prepared from the leaves of local cultivars of *Murraya koenigii*. Agar well diffusion was utilized to determine the zone of inhibition. Broth microdilution was employed to find out the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Anti-oxidant activity was analysed using 2,2-Diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay. The organic extracts displayed promising antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Ethyl alcohol extract presented highest inhibitory activity when compared to hexane and chloroform extracts. In relation to agar diffusion method, the ethyl alcohol extract displayed lowest MIC and MBC value of 12.5 and 25 mg ml^{-1} against *Staphylococcus aureus* and same MIC and MBC value of 25 and 50 mg ml^{-1} against *Escherichia coli* and *Pseudomonas aeruginosa*. The radical-scavenging activity of ethyl alcohol extract (IC_{50} = 30 µg ml^{-1}) is very close to that of the standard compound, gallic acid (IC_{50} value of 35 µg ml^{-1}). The results suggested that the local cultivars of *M. koenigii* carry beneficial health effects in terms of antimicrobial and anti-oxidant activities and could be used as a promising dietary supplement.

KEY WORDS: *MURRAYA KOENIGII*; ETHYL ALCOHOL EXTRACT; CARBAZOLE ALKALOID; ANTIBACTERIAL ACTIVITY; ANTIOXIDANT ACTIVITY

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INTRODUCTION

Spices and herbs which are used in food preparations traditionally are well known to contain several health promoting bioactive substances. Herbal medicines are used alternatively in many countries to cure many ailments (Kamboj, 2000). Herb based medicines are now marketed as nutraceuticals (health foods) (Brower, 1998). Indian Ayurvedic medicine is believed to be more than 5000 years old and it was well practiced and well recorded over the years (Garodia *et al*., 2007). Herbal drugs are generally used to prevent and treat various diseases and ailments or to support health and healing (De Smet, 1997). The World Health Organization (WHO) has also recognized the importance of traditional medicine and has set precise guidelines for the evaluation of the safety, efficacy, and quality of herbal medicines. In recent years the research on the medicinal properties of various traditionally used medicinal plants has gained considerable interest in order to ascertain their true medicinal values and also to assess their toxicity (Newman and Crag, 2007). Some drugs have been discontinued due to their toxicity, while others have been modified or combined with additional herbs to counterbalance side effects (Harish, 2001).

Bioactive principles of plants, also known as secondary metabolites exert a range of pharmacological activities in human beings besides serving as defense barriers in plants (Paul *et al*., 2015). They are responsible for therapeutic activities like hypoglycemic, anti-diabetic, anti-oxidant, anti-microbial, anti-inflammatory, anti-carcinogenic, anti-malarial, anti-cholinergic, anti-leprosy activities etc. (Makri and Kintzios, 2007; Negi *et al*., 2011). Common bioactive principles present in plants are, alkaloids, flavonoids, polyphenols, terpenoids, coumarins, tannins, saponins, anthocyanins and glycosides (Paul *et al*., 2015).

Additionally secondary metabolites also prevent spoilage and deterioration of food products Panghal *et al*., 2011). *Murraya koenigii* (L.) Spreng popularly known as curry leaf plant is a familiar spice used in India for its characteristic flavour and aroma. According to Wealth of India, it is also used in traditional Indian medicine as analgesic, febrifuge, stomachic, carminative and for the treatment of dysentery and skin eruptions. Recent studies on *M. koenigii* revealed its medicinal values like antidiarrhoeal, antimicrobial, hepatoprotective, radical-scavenging, hypoglycemic, and immunomodulatory properties (Maa *et al*., 2016). Phytochemical analysis of leaves, bark and root showed the presence of several bioactive constituents like carbazole alkaloids, β-carotene, polyphenols, terpenoids and coumarins (Malwal and Sarin, 2011; Gahlawat *et al*., 2014).

The pharmacological activities of *M. koenigii* as reported in different studies seem to exhibit a certain variation which is attributed mainly to the climatic differences among locations (Onayade and Adebajo, 2000). Therefore it has become a common practice to confirm that the local cultivars of *M. koenigii* possess the expected beneficial effects when consumed regularly. There are a few reports on phytochemical analysis and pharmacological activities of *M. koenigii* from India (Ningappa *et al*., 2008; Panghal *et al*., 2011; Malwal and Sarin, 2011; Biswas *et al*., 2012). But to my best knowledge, in recent years there are no studies on the antioxidant and antimicrobial activities of *M. koenigii* from Tamilnadu, a southern state of India. Therefore the present study on antibacterial and antioxidant activity of leaf organic extracts of local cultivars of *Murraya koenigii* from Tamilnadu was undertaken.

MATERIALS AND METHODS

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was procured from Sigma chemicals. Mueller Hinton agar and broth were purchased from Hi-Media, Mumbai. All other chemicals used were of analytical grade commercially available in India.The antibacterial activity of the organic extracts was tested against five bacterial strains. The only Gram positive bacterium included in the study was *Staphylococcus aureus* MTCC 96 (ATCC 9144). Four Gram negative bacteria used were *Escherichia coli* MTCC 901 (ATCC 13534), *Klebsiella pneumoniae* MTCC 109 (ATCC 15380), *Salmonella paratyphi* MTCC 735 and *Pseudomonas aeruginosa* MTCC 8291. All these bacteria were obtained from Microbial Type Culture Collection (MTTC), Institute of Microbial Technology, Chandigarh, India and they were maintained on nutrient agar. *M. koenigii* leaves were collected locally from various locations of Tamil Nadu, India and authenticated by Botanist. Fresh leaves were washed thoroughly and dried under shade till constant weight. The dried leaves were ground to powder in a mixer grinder.

100 g of shade dried *M. koenigii* leaves powder was extracted with hexane, chloroform and ethanol separately at room temperature for 2 days and then the solvents were evaporated using vacuum rotary evaporator at 50°C. The organic extracts thus obtained were dried at room temperature till constant weight and then stored in brown bottles at 4°C for further study.

Antibacterial activity of the three organic extracts obtained from *M. koenigii* was evaluated by agar well diffusion method on Mueller-Hinton agar (MHA). The bacterial strains were grown on MHA and suspensions were prepared to match 0.5 McFarland standard. 100 µl of the suspension was spread on MHA plates. Wells of 6 mm diameter were punched in the seeded agar using sterile borer. Test well were loaded with 50 µl extracts

(100 mg ml^{-1}) and negative control well loaded with same volume of solvent. Ampicillin and streptomycin were used as a positive control. The plates were incubated at 37°C for 24 h. The diameter of inhibition zones were measured in mm and results were recorded.

Minhibitory concentration (MIC) of the organic extracts was tested by the standard Clinical and laboratory standards institute (CLSI) methods (Wayne, 2008). MIC was the lowest concentration of the extract which gives no visible bacterial growth. Broth microdilution technique was used to determine the MIC (Elansary *et al*., 2012). The organic extracts from *M. koenigii* were serially diluted to obtain the dilutions, 200, 100, 50, 25, 12.5, 6.25 and 3.12 mg ml^{-1}. 100 µl from each dilution was transferred to respective wells followed by 90 µl of double strength Mueller-Hinton broth (MHB). Finally 10 µl respective bacterial suspensions were added to give final dilution of 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg ml^{-1} and the microtiter plate was incubated at 37°C for 24 h. Blank, positive and negative controls were in place. After incubation, the lowest concentrations of the extract, which did not show any visual growth of test organisms, were considered as MICs.

Minimum bactericidal concentration (MBC) of the organic extracts was also determined by the standard CLSI methods (Wayne, 2008). MBC was the lowest concentration of the extract which kills the initial bacterial inoculum. Broth microdilution technique was performed to determine the MBC. The extracts from *M. koenigii* were serially diluted to obtain the dilutions, 200, 100, 50, 25, 12.5, 6.25 and 3.12 mg ml^{-1}. 100 µl from each dilution was added to corresponding wells followed by 90 µl of double strength MHB. At last 10 µl respective bacterial suspensions were transferred to provide final dilution of 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg ml^{-1} and the plate was incubated at 37°C for 24 h. Blank, positive and negative controls were also included. After incubation, the wells which did not show any visual growth of test organisms were considered. 100 µl of the sample from those wells were transferred aseptically onto MHA, spread uniformly and the plates were incubated at 37°C for 24 h. After incubation, the concentrations at which no visible growth was seen were fixed as the MBC.

Free radical scavenging activity of the organic extracts from *M. koenigii* was evaluated by its ability to scavenge DPPH (Elansary *et al*., 2012). DPPH is a free radical, but stable in nature. The DPPH solution is initially violet in color which fades when antioxidants donate hydrogen. The change in color is monitored using a spectrophotometer and thus the DPPH free radical scavenging activity is calculated (Molyneux, 2004). A stock solution of 0.1 mM DPPH in ethanol was prepared. Test concentrations of organic extracts were made in the range of 50-250 µg ml^{-1} in ethanol. Gallic acid was used as standard. 1 ml of DPPH stock and 3 ml of test samples were mixed and allowed to react at room temperature for 30 min. The absorbance was measured at 517 nm using an ultraviolet visible spectrophotometer (Shimadzu, Japan). Lower absorbance of the reaction mixture indicated the higher free radical scavenging activity (Saikia and Upadhyaya, 2011). The percentage of free radical scavenging was calculated using the following Eq.

$$DPPH\ scavenging\ activity\ (\%) = \frac{(A0 - AT)}{A0} \times 100$$

where A0 is the absorbance of the DPPH solution and AT is the absorbance of the test or reference. The percentage of free radical scavenging was then plotted against the concentration and a regression equation was obtained to calculate the IC_{50} which is defined as the total antioxidants necessary to decrease the initial DPPH radical by 50% (Koleva *et al*., 2002).

All the experiments were done in triplicates. The values in the tables represent the arithmetic mean and standard deviation or mean. The standard error was within experimental limits.

RESULTS AND DISCUSSION

The organic extracts obtained from *M. koenigii* were less viscous in nature and brownish in colour. The yield of the extracts was estimated to be 7, 6.5 and 9.2 % (v/w) for hexane, chloroform and ethanol respectively. The yield in the present study was comparable to the yield reported in other investigations (Ningappa *et al*., 2008; Brind *et al*., 2014).

The results of antibacterial activity were presented in Table 1. Based on the diameter of zone of inhibition, the three organic extracts exhibited inhibitory activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Figure 1). *Klebsiella pneumoniae* and *Salmonella paratyphi* were found to be resistant except for chloroform extract which showed a very faint zone of inhibition. The organic extracts presented a good potential of antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* with the zones of inhibition well above standard antibiotic. Ethyl alcohol extract displayed highest inhibitory activity when compared to hexane and chloroform extracts. The results were comparable to that of previous studies in which the researchers reported that the various organic extracts of *M. koenigii* exhibited significant antimicrobial activity against both Gram positive and Gram negative bacteria (Panghal *et al*., 2011; Baskaran *et al*., 2011). This property is attributed to several carbazole alkaloids present in the organic extracts (Malwal and Sarin, 2011).

Table 1. Antibacterial activity of *M. koenigii* organic extracts by Agar well diffusion method

Test organism	Zone of Inhibition (in mm)			
	Hexane	Chloroform	Ethyl alcohol	Standard
Staphylococcus aureus	15.5±0.5	16.23±0.87	16.33±0.57	23.16±0.76
Escherichia coli	11.5±0.86	12.83±0.28	15.83±0.28	11.83±0.76
Klebsiella pneumoniae	0	7.83±0.76	0	11.86±0.8
Salmonella paratyphi	0	7.5±0.5	0	14.83±076
Pseudomonas aeruginosa	13.66±0.76	13.66±0.76	14.83±0.76	12.9±0.85

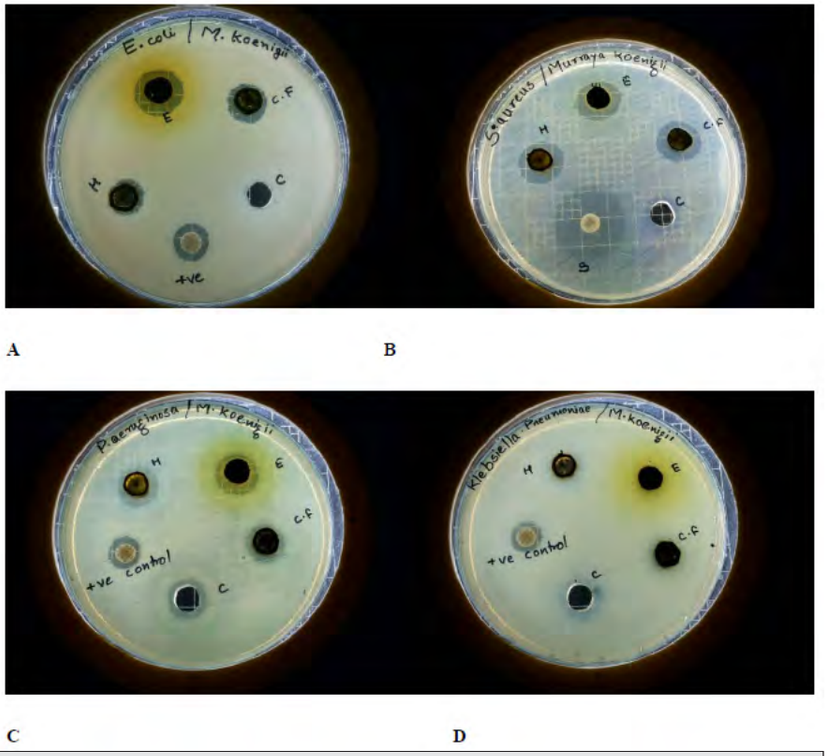

FIGURE 1. MHA plates exhibiting zone of inhibition of *M. koenigiiorganic* extracts against (A) *Escherichia coli*, (B) *Staphylococcus aureus*, (C) *Pseudomonas aeruginosa* and (D) *Klebsiella pneumoniae.*

The results of MIC and MBC of ethyl alcohol extract were summarized in Table 2. Relative to the results of agar diffusion method, the extract displayed lowest MIC and MBC value of 12.5 and 25 mg ml^{-1} respectively against *Staphylococcus aureus*. The extract produced same MIC and MBC value of 25 and 50 mg ml^{-1} against *Escherichia coli* and *Pseudomonas aeruginosa*. The results are equitable to the results presented by Panghal et al. in which the organic extracts prepared from the leaves of *M. koenigii* manifested slightly better MIC values against the clinical isolates (Panghal *et al.*, 2011). Zinc oxide

Table 2. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *M. koenigii* organic extracts

Test organism	MIC (mg ml^{-1})		MBC (mg ml^{-1})	
	Test	Standard	Test	Standard
Staphylococcus aureus	12.5	3.12	25	6.25
Escherichia coli	25	25	50	50
Pseudomonas aeruginosa	25	25	50	50

Table 3. Antioxidant activity of *M. koenigii* organic extracts measured in term of DPPH radical scavenging ability

Concentration (µg ml-1)	DPPH scavenging activity (%)				IC50 value (µg ml-1)
	Ethyl alcohol	Hexane	Chloroform	Gallic acid	Gallic acid = 35 Ethyl alcohol = 30 Hexane = 22 Chloroform = 27
20	12	9	9	13	
40	23	16	21	28	
60	35	25	33	42	
80	48	34	42	55	
100	62	45	54	68	

nanoparticles synthesized using the leaf extract of *M. koenigii* displayed almost similar results to the present study (Elumalai *et al*., 2015). Antioxidant protein isolated from the leaves of *M. koenigii* also exhibited comparable results (Ningappa *et al*., 2010). The variation in the antimicrobial activity of *M. koenigii* organic extracts specified in various studies may be due to the differences in chemical composition which can result from the climatic differences among locations (Paul, 2015). Though several compounds like tocopherol, β-carotene, lutein and alkaloids (Khanum *et al*., 2000) are reported in the leaf organic extracts, carbazole alkaloids are accounted for the antimicrobial activity (Ramsewak *et al*., 1999).

The results of free radical-scavenging ability of organic extracts of *M. koenigii* measured by the DPPH assay were given in Table 3. Ethyl alcohol extract displayed highest radical scavenging activity with the IC_{50} value of 30 µg ml^{-1} than the rest two extracts. The radical-scavenging activity of ethyl alcohol extract is very close to that of the standard, gallic acid exhibiting the IC_{50} value of 35 µg ml^{-1}. This is in agreement with other studies on anti-oxidant activity of leaf extracts of *M. koenigii* (Rao *et al*., 2007; Ningappa *et al*., 2008; Kusuma *et al*., 2011). The antioxidant activity of organic extracts from *M. koenigii* is attributed to the presence of polyphenolic compounds and carbazole alkaloids (Rao *et al*., 2007; Garodia *et al*., 2007). Ethyl alcohol extract exhibited greater radical-scavenging activity than the other two extracts and this can be inferred as ethanol is effective in extracting polyphenols (Singh *et al*., 2011).

CONCLUSION

As substantiated in the study, the ethyl alcohol extract from the leaves of *M. koenigii*, grown locally to fulfil culinary needs of Indian cuisine displayed excellent antimicrobial activity against both Gram positive and negative bacteria and also exhibited a quality free radical scavenging activity. Climate of the location and growing season greatly influence the chemical composition of *M. koenigii* and thus have an impact on the biological activities. The above study confirmed that the local cultivars of *M. koenigii* possess beneficial health effects in terms of antimicrobial and anti-oxidant activities. Consequently, local cultivars of *M. koenigii* could be effectively used as spice and dietary supplement, however further study on molecular mechanism behind the role of various constituents on radical scavenging and antimicrobial inhibition needs to be done.

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Effect of premarital counseling on shyness and expectations from marriage among medical science students

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ABSTRACT

The period before from marriage is the one of the sensitive and critical periods in life and also is very influential period in preventing marital problems after marriage. The aim of this study is to investigate the effect of premarital Counseling on shyness and expect from marriage among students of Medical Sciences, Torbat Heydariyeh, Iran. This quasi-experimental study was conducted on 30 single students of Medical Sciences in Torbat Heydariyeh Iran. The assessment tools was shyness and expect from marriage scales that were complete d in the pre-test and post-test by participants. The intervention group participated in 8 sessions of premarital Counseling and the control group received no intervention. Data were analyzed using descriptive and Analytic statistics. The mean age of participants in control and intervention groups was 19.33±0.61 and 19.92±1.07 years, respectively. There was no significantly different between background variables of two groups ($p>0.05$). In the control group there was no significant difference in shyness and expect from marriage, before and after the study, but there was a significant difference between two mentioned variables in the intervention group ($p<0.05$). According to the results, it is suggested that premarital Counseling used for students before Choice of spouse until to reduce the disturbing factors in spouse selection process. Premarital Counseling improves shyness, cynical and idealistic expectations from marriage.

KEY WORDS: PREMARITAL COUNSELING, SHYNESS, MARRIAGE, EXPECT FROM MARRIAGE

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INTRODUCTION

Formal legal marriage is a contract that is closed between men and women and Considers for each person special place and role (Houlgate, 2017). In recent years the use of pre-marital counseling program to strengthen marriage is common. Premarital counseling for couples preparation and prevent dissatisfaction and failure in marriage has a significant impact and people learn how to be a successful and stable marriage, so premarital counseling have aspects of preventive, educational and therapeutic (Parhizgar et al., 2017). The research shows that traditional counseling before marriage is not benefiting for married life and had not a positive impact on the stability of marriage (Moeti et al., 2017).

Premarital period is one of the sensitive and critical points in life and is a very effective period in the continuity of life and prevention of marital problems after marriage (Vural & Temel, 2009). In post marital period, one of the factors that cause a basic change in the process of marriage and its path is expect of people from marriage, roles and marital needs (Bagarozzi, 2014). According to the findings of researchers, having optimum belief, attitude and expect from marriage is the best and most important capital in marriage and guarantees more happiness of people in the common marital path. Expects and beliefs of people from marriage affect the perception of people from each other and probability of marriage or non-marriage and determination of marriage time (Parrott & Parrott, 2005).

Expectations from marriage is a set of realistic, idealistic and cynical expects that shows the beliefs of people to triple fields of marriage namely intimacy, agreement and equality. expect from marriage shows the expects of people from the level of intimacy and closeness to the spouse, type of personality and attractiveness of future spouse, type of activities inside and outside of family, and the rate of responsibility and equality of couples in the affairs related to home and children (Nilforooshan, Abedi, Ahmadi, & Navidian, 2011). Expectations from marriage in people in premarital period has a significant relationship with married life and life satisfaction like the level of intimacy, distance, division of labor, and the nature of the relationship between husband and wife with the main family (Holman, 2006). Theoretically, the primary experiences of the persona and relationships, whether as the observer of a relationship or participant in it, has a main role in the formation of expect from marriage. According to the Bowlby's attachment theory, the primary experiences of person in relationship with the beliefs and expects of people from the next relationships (Mikulincer & Shaver, 2007).

The study of expectations from marriage in premarital period is very important so that the researchers in a study examined and graded the areas of couples problems and mentioned the unrealistic problems and expect from marriage and lack of intimacy and interest as the most important marital problem (Mazlomi, Dolatshahi, & Nazari, 2010). One of the other important issues that can affect the decisions of people in marriage is shyness. Shyness is associated with several distinct behavioral antecedents and biological correlates across development, including early behavioral inhibition and neuroendocrine dysregulation, (Poole et al., 2017).

Shyness has become the main concern of different societies during the recent years and has become an important issue, so that it has led the researchers toward understanding this problem (Koydemir & Demir, 2008). Shyness is considered as a kind of social anxiety that is defined with the features like anxiety in front of an audience, distress and worry and shyness from being seen by others (Botvin & Kantor, 2000). Paying too much attention to the words and behaviors of oneself and fear of interaction with others, fear of not being confirmed by others and worry of being judged is the main features of shy people. These people make conditions stressful and while being exposed to new situations or new people with illogical and automatic thoughts and usually avoid asking questions or even answering the questions of others (Shahri et al., 2014).

The study results of Afrooz show that the increase of the people who have problem in relationships with others, those who have fear of social contacts and are shy, the young people who do not know what to say and how behave when they enter a group, sense of inability to talk to people and lack of cooperation with them, depression and social isolation and finally rejection by society (Afroz, 2002). Studies show personality and therefore also shyness is influenced by various sociodemographic variables, social roles and important events, which affect stability and lead to changes in personality (Van Zalk, Lamb, & Jason Rentfrow, 2017). Also Poole, Van Lieshout & Schmidt believe emerging evidence suggests that conflicted shyness (combination of shyness and sociability) places individuals at risk for maladjustment in childhood, adolescence, and emerging adulthood (Poole, Van Lieshout, & Schmidt, 2017).

Since expectations from marriage is one of the most important factors to guarantee post marital satisfactions and on the other hand shy people are susceptible to make wrong decisions for marriage, without a doubt these two areas of evaluation have an important role in the guarantee of right decisions for marriage. For this purpose, today it is attempted to use the premarital programs for single people. Premarital counseling is a new approach that presents some education in order to prevent the post marital dissatisfaction (Salarvand, Bahri, Heidary, & Khadive, 2011).

The experts of family area are suing about the efficiency of premarital counseling and teach people how to have a successful and durable married life (Fathzadeh et al., 2008). To final purpose of premarital counseling is to help the strength and stability of marriage, the reduction of divorce rate and promotion of marriage quality. Premarital counseling program with the study of effective factors in successful marriage and the role of these factors in the supply of adjustment and marital life satisfaction deal with some approaches to select the appropriate spouse and successful marriage and also prevent frustration in married life and study of its dimensions and factors through the mutual understanding of spouses, understanding the needs of both sides, mutual understanding, love, kindness and mutual commitment (MirMohammad Sadeghi, 2010). Although the effectiveness of premarital counseling to promote the level of life has been examined but its effect on expect from marriage and shyness of single students has been les studied. Thus, the present study aims to investigate the effect of premarital counseling on shyness and expect from marriage among students of Medical Sciences, Torbat Heydariyeh, Iran.

MATERIAL AND METHODS

The present study was a quasi-experimental intervention with pre-test, post-test and control group. The statistical population includes all single and new arrival students of Torbat Heydarieh university of medical sciences in 1394 (n=110). After taking the licenses from Torbat Heydarieh university of medical sciences and taking conscious consent of the participants, 30 single students were selected by random-stratified method and were placed in the intervention group and control group (groups of 15) by random allocation method. The inclusion criteria of participants to the study were gaining high score and very low score in the scale of expect from marriage and gaining a score more than 67 in shyness test. At the beginning of the study, the scale of expect from marriage and shyness scale was completed by the participants of intervention group and control group. Premarital counseling was performed in 8 sessions of 2 hour for the intervention group. During this time, the control group did not receive any education. The summary of activities in intervention sessions are as follows:

The first session; teaching concepts familiar, attractive, familiar theories, advantages and limitations of familiarity, ten mistakes in the process of dating and matchmaking.

Second session; Training love triangle theory, the elements of love, the effect of the issue on love, choose love and complete unfinished love children and facilitate donors.Third session; Life skills training including assertiveness skills, effective communication skills, assertiveness, stress management, anger management, etc. Fourth Session; Training process of mate selection, right and wrong reasons to marry, false expectations of marriage, people who are not suitable for marriage, personality disorder and inappropriate for marriage, good relationships that are not marriage, married warning signs, criteria of successful marriage. Fifth Session; Defining engagement, the marriage, the difference candidate with familiarity, forced marriage, commitment concepts, borders and boundaries, and the time of engagement. Sixth Session; the Marriage period, definition of marriage period, difference between marriage and engagement, requirements and functions of marriage period. Seventh session; Issues related to family, wife, threads between family, family issues related to the main economic issue, dowry, dowry, etc., division of labor in the process of marriage and expectations associated with these conditions and preparing participants for sessions. Eighth Session; Informing students about early marriage and its challenges, build confidence, balance between the need for affiliation and need for independence, competitors, marriage, having children and sessions. After the end of training secessions, the participants of intervention and control groups completed the questionnaires again. The measurement tool was the standard questionnaire of Jones and Nelson expect from marriage and Stanford shyness.

Marriage expectation scale (MES) includes 40 items and 3 sub scales of cynical expect, realistic expects and idealistic expects. Its scoring range was graded according to Likert 5-point scale as totally disagree with 1 to totally agree with 5. The minimum score in this scale is 40 and maximum score is 200. Lower scores show cynical expect from marriage and higher scores show idealistic expect from marriage. Average scores show realistic expects (Nilforooshan et al., 2011). Jones and Nelson reported the reliability of questionnaire for the total scale by Cronbach's Alpha as 0.79 to 0.80 and Dilon as 0.80 (Dillon, 2005; Jones & Nelson, 1997). The reliability of this questionnaire in the present study was obtained as 0.74.

Stanford shyness scale includes 40 items that was made in Stanford University and measures the shyness of people. Stanford shyness scale is in all questions with Likert 4-point scale including never (code 1), sometimes (code 2), often (code 3), and always (code 4). The minimum score is 40 and maximum score is 160. The score from 40 to 60 shows low shyness. 67 to 100 average shyness and more than 100 shows high shyness. In the study of Najaf Abadi et al., the validity of shyness was 0.74 and its reliability was obtained as 0.87 by Cronbach's Alpha method (Najaf Abadi et al., 2013). In the

present study, the reliability of the tool was confirmed as 0.77 for Cronbach's Alpha. The statistical data of the study were analyzed by using SPSS statistical software version 21.

RESULTS AND DISCUSSION

The man age of participants in the control group was 19.33±0.61 and in intervention group was 19.92±1.07. In the control group 7 subjects (47.7%) and in intervention group 6 subjects (40%) were women. The frequency of medical emergencies was 5 subjects (33.3%), midwifery 7 subjects (46.7%) and anesthesiology was 3 subjects (6.7%) that this number is equal in both groups of control and experimental. In terms of economic status, the maximum frequency was related to average economic status with 10 subjects (66.7%) in control group and 8 subjects (53.3%) in the experimental group. The use of Chi-2 statistical tests for the variables of gender, economic status, education and independent T-test for age showed that there is not a significant difference between the mentioned variables in the two groups of control and experimental (p>0.05). It means that the two groups were homogenous in terms of the mentioned variables.

To compare the amount of shyness between the two groups of control and intervention before and after intervention, the Mann–Whitney test was used. This test did not show any significant difference between the two groups before intervention in terms of shyness in students. (p=0.96) But after the intervention, there was a significant difference between the two studied groups in terms of shyness (p=0.01). To study the two groups before and after the intervention of the Wilcoxon test was used. The results show that in the control group after the intervention, there was no statistically significant difference in the degree of shyness (p=0.31). But there was a statistically significant difference in intervention group (p=0.01). So that at the beginning of the study, 4 subjects had severe shyness (26.7%) and 9 subjects had average shyness (60%) in the experimental group but after the intervention, the number of shyness reduced to 0 subjects (0%) and average shyness reduced to 6 subjects (40%) (Table 1).

The second hypothesis of the present study claimed the significant difference between the intervention group and control group in terms of expect from marriage. The obtained score of any sub scale, the relevant questions were added together and since the score range for each sub scale is different, each range was considered as 0 to 100 for the better understanding of scores range. The results of kolmogrov-smirnov showed that expect from marriage and its sub scales (realistic expects, cynical expects, idealistic expects) are normal in the control and experimental group before and after the intervention (p>0.05). Accordingly, the parametric tests were used for analysis.

The results of independent t-test showed that the intervention and control group had no significant difference at the beginning of the study in terms of the score of expect from marriage (p>0.05). It shows the assimilation of sample in the two groups according to the intended variables, but these were a significant difference after the performance of premarital counseling for intervention group (p<0.05) (Table 2).

To study the two groups before and after the intervention, the Paired t-test was used. The results of paired T-test showed that the mean score of expect from marriage in students after holding premarital sessions in intervention group (p<0.05). This difference was not seen in the control group. (p>0.05) (Tables 3, 4 and 5).

According to the obtained results, premarital counseling has a significant effect on the shyness of students. Bashirpoor et al in a study compared the effect of group counseling with cognitive – behavioral approach and existential approach on the decrease of shyness in students. Both approaches had a significant effect on the shyness of male students but in their study, the interven-

Table 1. The comparison of shyness between the participants before and after the intervention in the two groups of control and experimental[a]

Variable	time	Variable classes	Control	Experimental	*P*[b]
shyness	Before intervention	Weak	1(6.7)	2 (13.3)	0.96, z=-0.05
		Average	11 (73.3)	9 (60)	
		Severe	3 (20)	4 (26.7)	
	After intervention	Weak	2 (13.3)	9 (60)	0.01, z=-2.7
		Average	11 (73.3)	6 (40)	
		Severe	2 (13.3)	0 (0)	
	P-value[c]		0.31, z=-1	0.01, z=2.49	

[a]Values are expressed as N (%).
[b]Mann-Whitney test results
[c]Wilcoxon test results

Table 2. The comparison of the mean score of realistic expect from marriage in participants before and after intervention in the two groups[a]

variable	time	Control	experimental	*P*[b]
Realistic expects	Before intervention	51.17±7.46	49.31±6.21	0.46, t=-1.67
	after intervention	49.31±7.39	68.23±14.10	<0.001, t=-2.72
	P-value[c]	0.08, t=1.47	<0.001, t=4.56	

[a]Values are expressed as mean (SD).
[b]Independent t-test results
[c]Paired t-test

Table 3. The comparison of the mean score of idealistic expect from marriage in participants before and after intervention in the two groups[a]

variable	time	Control	experimental	*P*[b]
Idealistic expects	Before intervention	70.95±15.06	75.83±14.33	0.46, t=-0.9
	after intervention	73.45±12.51	59.16±18.38	0.02, t=-2.40
	P-value[c]	0.09, t=-1.78	0.006, t=3.25	

[a]Values are expressed as mean (SD).
[b]Independent t-test results
[c]Paired t-test

tion method of group counseling was cognitive-behavioral and existential while in the present study the intervention method was premarital counseling (Bshirpoor, Salimibejestani, & Farahbakhsh, 2013). Also, Rahmani and BaniAsadi in a study examined the effectiveness of teaching non-verbal relationship on the shyness of students and the obtained results showed that these educations have decreased the shyness of students in physiological and behavioral dimensions. However, the intervention method in the study of Rahmani et al. was different from the present study (Rahmani & BaniAsadi, 2013).

To explain the obtained results, the attitude of Gudarzi et al can be mentioned. They believe that the science of life skills education as a preventive method can have positive and useful effects on the increase of emotion control, coping with stress, high self-confidence, and increase of self-concept, increases of self-esteem, better problem solving and interpersonal conflicts that lead to the reduction of shyness (Goudarzi, MakvandHosseini, Rezai, & Blotbngan, 2013).

In the present study, one of the discussions that were taught in sessions with students was teaching life skills such as establishing effective relationships,

Table 4. The comparison of the mean score of cynical expect from marriage in participants before and after intervention in the two groups[a]

variable	time	Control	experimental	*P*[b]
Cynical expects	Before intervention	65±13.65	73.6±9.02	0.06, t=3.25
	after intervention	57.73±8.86	45±8.01	<0.001, t=-6.21
	P-value[c]	=0.09, t=-1.76	<0.001, t=4.29	

[a]Values are expressed as mean (SD).
[b]Independent t-test results
[c]Paired t-test

Table 5. The comparison of the mean score of general expect from marriage in participants before and after intervention in the two groups[a]

variable	time	Control	experimental	*P*[b]
Expects from marriage	Before intervention	54.41±24.11	65.91±11.22	0.13, t=-1.56
	after intervention	61.41±11.54	54.29±4.81	p<0.001, t=-4.22
	P-value[c]	0.1, t=1.64	0.03, t=2.2	

[a]Values are expressed as mean (SD).
[b]Independent t-test results
[c]Paired t-test

self-assertion, stress management and assertiveness and this education can be effective in the reduction of shyness in students. People attend the society with more self-confidence when they are equipped with social skills, so they create successful relationships and this success increases their self-confidence and anxiety to attend in front of an audience (Dokanei Fard, 2007). Mahruz Yadak and Hatami also confirmed the effect of teaching life skills on shyness and believe that people can make better decisions and cope with emotions like fear, anger, shyness or sadness in an appropriate way when they are equipped with these teachings (Mehroz Yadak & Hatami, 2010). Also, premarital counseling improved the cynical and idealistic expects in most students. According to the results of the present study, premarital counseling could reduce cynical and idealistic expects and increase realistic expects. Before the intervention, the average realistic expects of students was low but after premarital counseling, the average realistic expects increased and this result was useful. The average cynical expects of students was high before the intervention and significantly decreased after the intervention. Also, the average idealistic expects of the intervention group had a significant difference before and after the intervention and this difference was not observed in the control group that did that did not receive any intervention.

The results of this study are in line with the study of Omidvar et al. they examined the effect of premarital Counseling on expects from marriage of students and the results showed that the counseling have a significant effect on expects from marriage (Omidvar, FatehiZadeh, & Ahmadi, 2009). Also, Sharp and Ganeng in their study proved the effect of systematic education on unrealistic and romantic expects of university students and showed that the students significantly adjusted their unrealistic and romantic expects due to education (Sharp & Ganong, 2000). To explain the obtained result, the content of the sessions of the present study can be mentioned. In the present study, one of the issues that were studied in group sessions was the issues that were studied in group sessions was the study of the role of wife and husband, expect of participants in the study from their future spouse, expects and objectives of participants from marriage and creating family. Negative reasons for marriage including space away from inappropriate family environment, economic problems and positive reasons for marriage like the need to love and intimacy, being parent and the need to accompany were clarified. The subjects were explained that cynical or unrealistic expects lead to frustration and the frustration due to the non-satisfied expects causes anger, and dissatisfaction from marital life (Ghahari, 2012).

Counseling can be a reason for the changes of idealistic and cynical expects from marriage. Also, in sessions, the technique of exploration was used to clarify the meaning of expect from marriage in people and their beliefs. The double standard technique was used to change the wrong expects. So that, the participants were asked: what will you say if your close friend has a similar selection like you and rely on his/her spouse to meet all his/her expects?

Double standard technique makes people look at their problem in such a way as if it is the problem of someone else. Though this method, the participants found that expects from marriage is unrealistic. In general, premarital counseling led to the improvement of shyness and expect from marriage.

CONCLUSION

According to the study results, it is suggested that premarital counseling is used before the selection of spouse in order to reduce nuisance factors in the process of choosing spouse. Premarital counseling could reduce shyness, idealistic and cynical expects from marriage by providing information on the field of risky marriages, relationships before marriage, barriers of appropriate process of information, teaching how to identify cognitive distortions and methods of challenging the false beliefs and effective relationships.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interests regarding the publication of this article.

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Exploring climate change over Khazar Basin based on LARSE-WG weather generator

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ABSTRACT

The aim of this paper is to analyze climate change over Khazar Basin for the next decades. Khazar Basin is considered as one the most important one in Iran due to its specific climatic conditions. The experiment of climate change across the basin was conducted using 21 synoptic stations by the application of LARS-WG weather generator, the years from 1992 to 2015 were selected as the base period and the analyses were carried out to estimate climate change over the 2046-2065. The analyses for future climate change then were compared to the base period. The results revealed that in the future period, on average precipitation will decrease -37.1 mm and min and max temperature will increase 3.8 and 3.2 ^{o}C respectively compared to the base period. Therefore it could be deduced that climate change can have adverse effects on the Basin and some adaptive steps must be taken to reduce the bad effects accordingly.

KEY WORDS: CLIMATE CHANGE, LARSE-WG, KHAZAR BASIN, PRECIPITATION, TEMPERATURE

INTRODUCTION

Developing countries are vulnerable to climate changes, mainly due to their limited adaptive capacities in dealing with extreme events (Pouliotte et al., 2009). the main findings from the latest report on climate change and a special report on extreme events from the Intergovernmental Panel on Climate Change show a greater consensus about a likely increase in the frequency and intensity of heavy precipitation events over land areas since 1950 (IPCC). Therefore, it is of great importance to predict climate change for the future decades to deal with its possible effects over different geographical territories. In the past years many investigations have been conducted to reveal climate change for different temporal resolutions and also for different parts of the globe. The effects of climate change has been simulated in 12 rivers of India, the authors have concluded that under a scenario of climate change a general reduction in the quantity of the available runoff will occur and the intensity of floods

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in various parts of India may get deteriorated (Gosain et al., 2006). The results of an investigation also show that between 1995 and 2025, the extents of the regions that will be affected by severe water stress will increase and this condition is especially true for Southern and Western Africa and South Asia (Alcamo et al., 2000). In a research it was found that based on ENSEMBLES anthropogenic climate-change (ACC) global simulations and the Climate version of the Local Model (CLM) precipitation is expected to be critically decreased in three selected region of Greece (Paparrizos et al. 2016).

In another study it was revealed that over the Central Asian region, the aridity is expected to increase (Lioubimtseva and Henebry, 2009). In another research work it was found that for the periods from 2010 to 2040 and 2070 to 2100 and based on the CGCM 3.1 dry regions of the Iran will get less precipitation (Abbaspour et al., 2009). The findings of Arnell and Gosling (2016) revealed that under the climate model (HadCM3 and SRES A1b), in 2050 the current 100-year flood would occur at least twice as frequently across 40 % of the globe, approximately 450 million flood-prone people and 430 thousand km2 of flood-prone cropland would be exposed to a doubling of flood frequency, and global flood risk would increase by approximately 187 % over the risk in 2050 in the absence of climate change.

Zhang et al. (2016) estimated stream flows in the Xin River Basin, China based on climate change scenarios downscaled from different GCMs (BCC-CSM1.1, CanESM2, and NorESM1-M) under three Representative Concentration Pathways (RCPs). The ensemble average of streamflow in GCMs demonstrated that many RCPs significantly decrease from May to June but increase from August to September relative to the baseline period. The goal of this investigation is to reveal the changes of temperature and precipitation over the study region. As rain fed agriculture is very common in the basin the findings of the current paper could assist policy makers to have a better picture of climate of the study region for the next decades.

MATERIAL AND METHODS

STUDY REGION

Khazar Basin is geographically located in the north parts of Iran with a very diverse climate. The Alborze Mountains which are extended from west to east have created two distinct climates in the region. The north parts of the Basin receive a lot of precipitation throughout the year while the southern counterpart receives much less precipitation as the humidity is often confined to the northern areas of the Basin. The climate of the north part is mild and humid but the western parts of the region

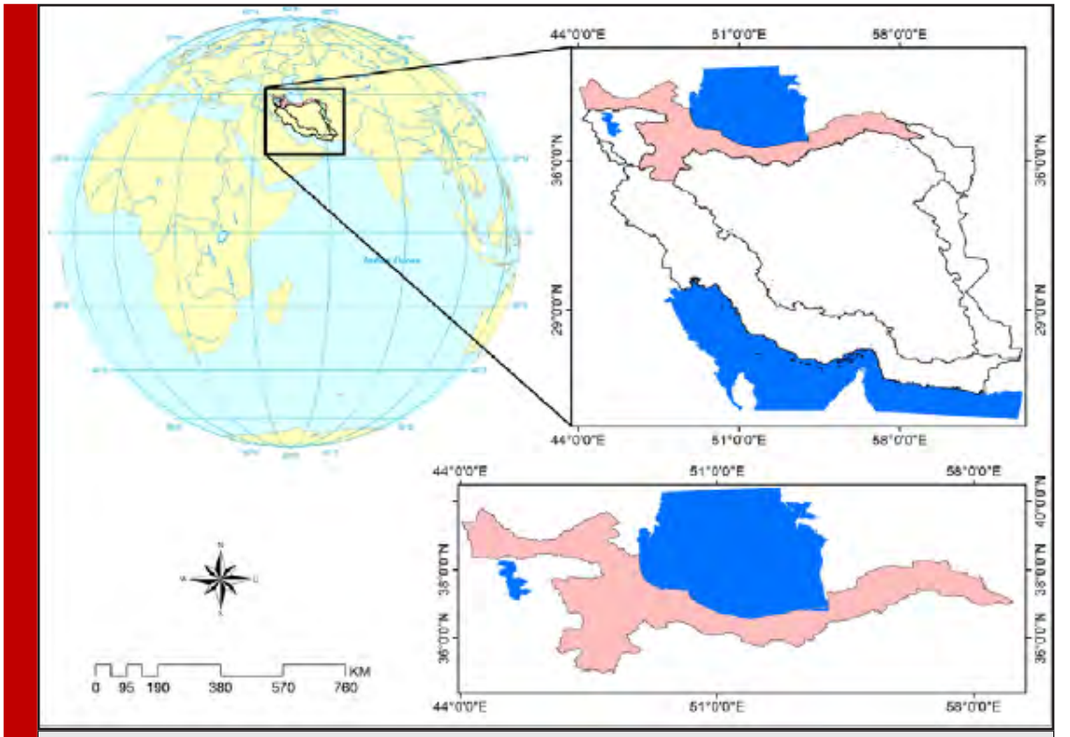

FIGURE 1. General location of Khazar Basin.

have a cold climate. The general location of the Basin has been depicted in Figure (1).

MATERIALS AND METHODS

In this paper, the daily precipitation, min temperature and max temperature data of 21 synoptic stations across the Basin were applied from 1992 to 2015. The applied stations are operated by Islamic Republic of Iran Meteorological Organization (IRIMO) and are presented in text files by the IRIMO. In order to ensure a valid climate change analyses it is important to examine the accuracy of the applied data, so the climate variables of all the stations have been checked for any possible outliers in the time series. Despite the fact that the resolution of General Circulation Models (GCMs) has been significantly increased but they still cannot predict meteorological outputs for small scales. Therefore, some models have been created to downscale the GCMs output. LARS – WG is a model which is used to downscale the GCMs output. In this investigation the LARS – WG version 5.5 was utilized. This version includes different GCMs data and the BCM2 which is one the GCMs has also been included in the model. LARS – WG utilize separate semi-empirical distributions for each of variables including min, max temperatures and precipitation and this downscaling method has been used in several studies (Semenov et al., 1998; Qian et al., 2004; Babaeian and Kwon, 2005; Lawless and Semenov, 2005; Khan et al., 2006).

In the next step to prepare the data to run the model, the data of each station were transferred into the format of the LARS- WG weather generator. The observed daily max temperature, min temperature and precipitation series for 1992–2015 at 21 stations are used as inputs to the LARS- WG weather generator and then the weather series of the variables are generated for each site. Using the BCM2 general circulation model with emission scenario of SRA1B we simulated the climate variables of max temperature, min temperature and precipitation for the future period of 2046-2065 over the Basin. Then we computed the Basin wide average of min, max and precipitation for the observed period and also for the future period based on the obtained results from the model. Using the Surfer 12 software package, all the three climate variables were then interpolated over the Basin and mapped.

RESULTS AND DISCUSSION

The calculated observed min temperature for the Basin indicates that the central parts of the Basin are the warmest areas compared to the other regions and in some parts the annual temperature is nearly 13 °C, the west and east parts of the Basin are cold parts and in some areas in the north-west the min temperature falls below 0 °C (Figure 3). The simulated annual min temperature indicates that compared to the based period there will be increase in temperature (Figure 4) and in all areas of the Basin min temperature will increase in various rates. The highest rate of increase is seen over the north-west parts of the Basin that are considered to be the cold and mountainous regions (Figure 5). In these regions the temperature increase is up to nearly 7 °C but in central areas the increased temperature is less evident and it is generally less than 2 °C.

For the observed period the max warmest temperatures in the Basin are generally seen over central to the east regions while the cooler max temperatures are over north-west of the Basin. The max temperature is below 15 °C in the coolest parts but in the warm areas, the max temperature exceeds 21 °C (Figure 6). In the future period the max temperature will increase for entire of the Basin (Figure 7) and the temperature in some areas will exceed 24 °C. The map of difference of max temperature (future – base) indicates that north areas of the Basin from north-west to north-east will experience the

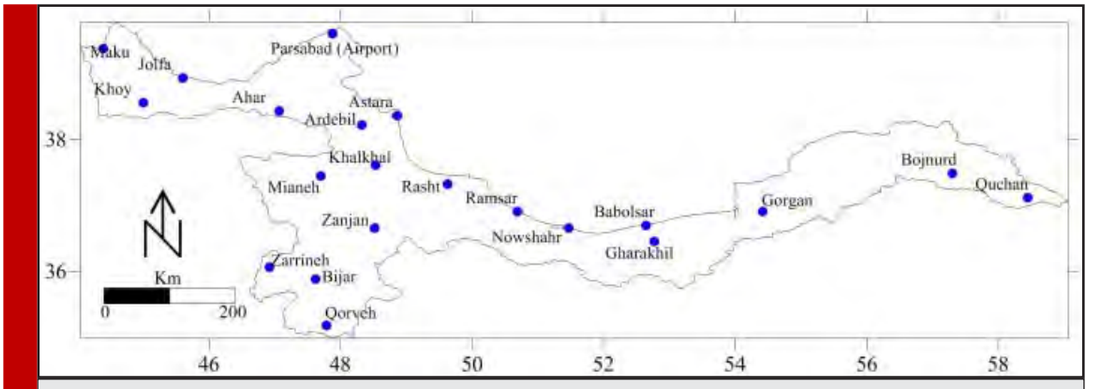

FIGURE 2. Location of the stations used in this study

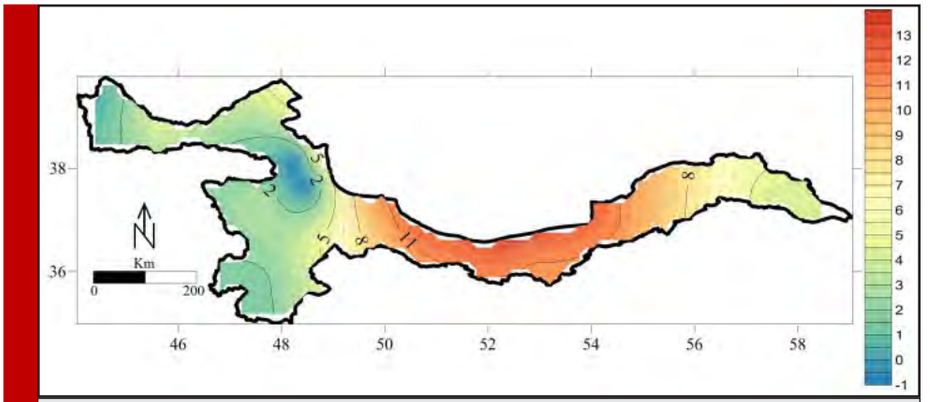

FIGURE 3. Map of observed min temperature (°C) for the based period (1992-2015).

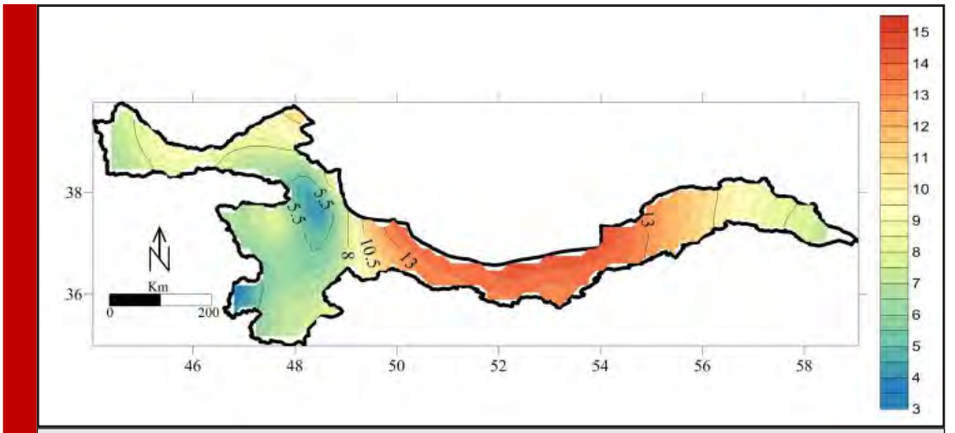

FIGURE 4. Map of simulated min temperature (°C) for the future period (2046-2065).

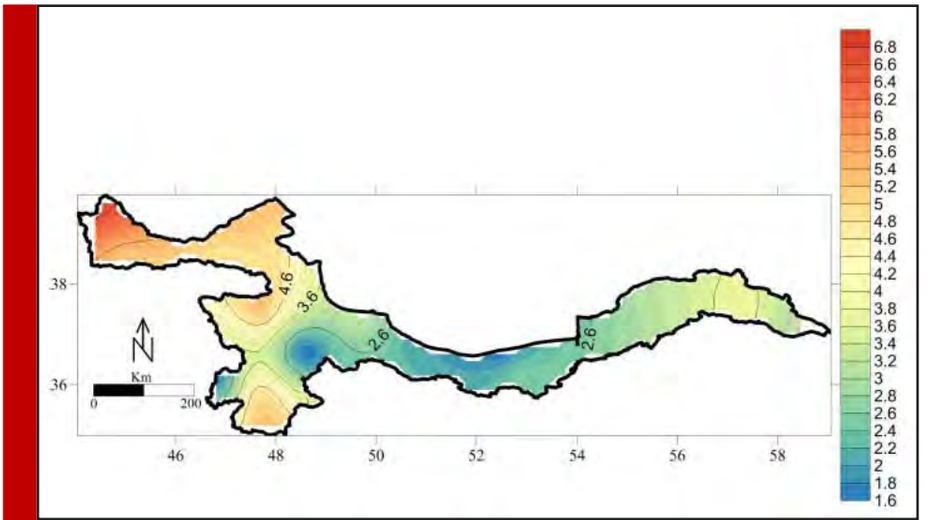

FIGURE 5. Map of difference of min temperature (future-base)

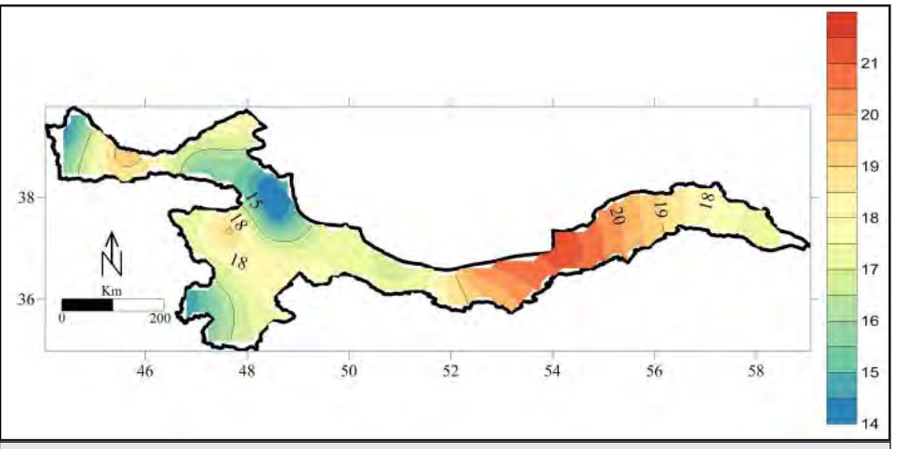

FIGURE 6. Map of observed max temperature (°C) for the based period (1992-2015).

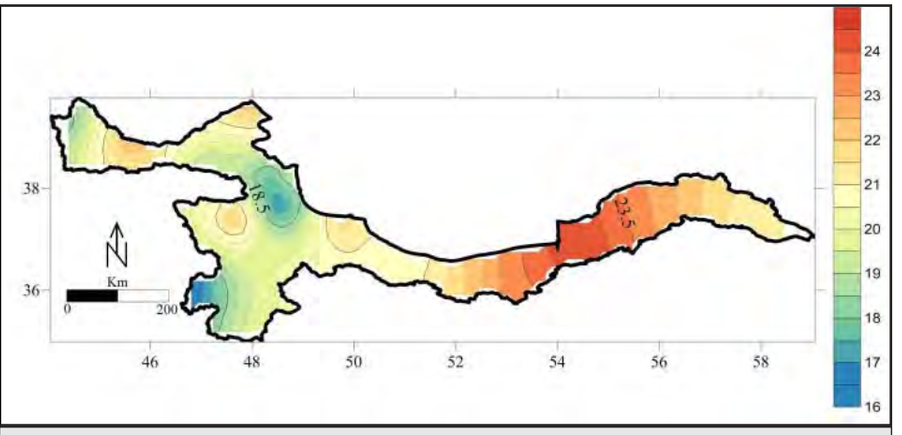

FIGURE 7. Map of simulated max temperature (°C) for the future period (2046-2065).

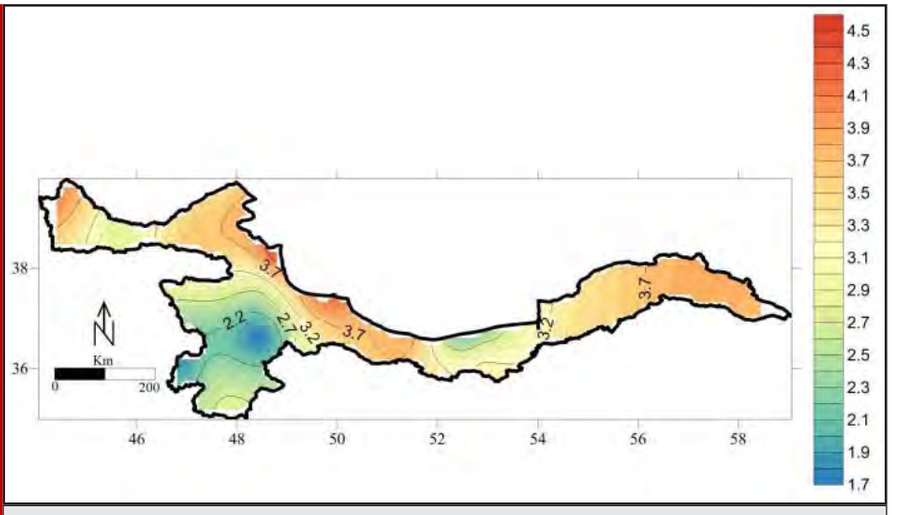

FIGURE 8. Map of difference of max temperature (future-base)

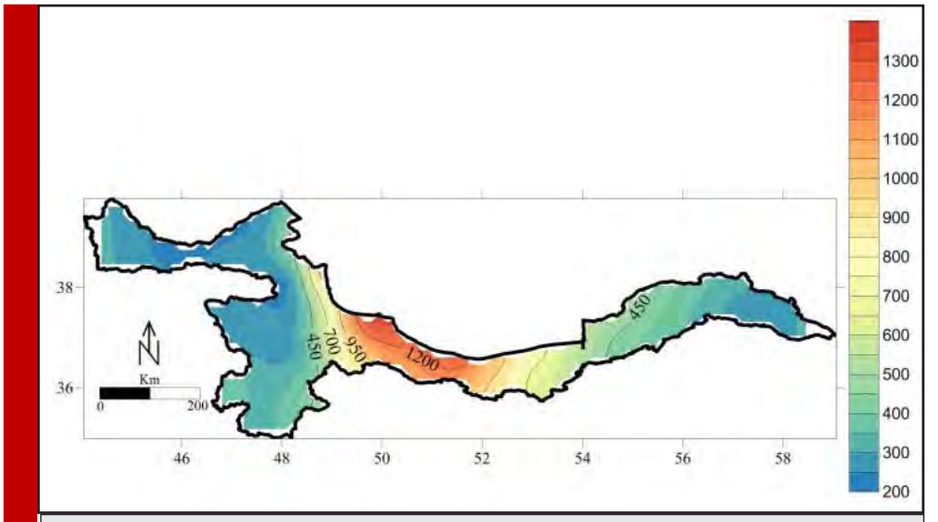

FIGURE 9. Map of observed precipitationfor the based period (1992-2015).

highest rate of temperature increase, in these regions the temperature can possibly increase up to 4.5 °C but this rate is far less evident in south-west of Basin, in these areas temperature increase is below 2 °C in general (Figure 8). The overall rate of temperature increase for the entire of the Basin is 3.2 °C.

Owing to the complex topography of the Basin, the amount of precipitation is also highly variable across the Basin, in the central north parts, the annual precipitation is very significant and it's due to the proximity to Caspian Sea. In some parts the annual precipitation exceeds 1300 mm on average (Figure 9). The map of simulated precipitation for the future period indicates that many areas of the Basin will experience reduction in precipitation (Figure 10). The rate of decrease is various from region to region, the highest reduction is identifiable over central to the west parts with the average reduction of up to 140 mm while some areas from central to the east have an increase amount of precipitation compared to the base period with an increase of up to 40 mm (Figure 11). In general for the future period, the precipitation will decrease 37.1 mm in comparison to the base period.

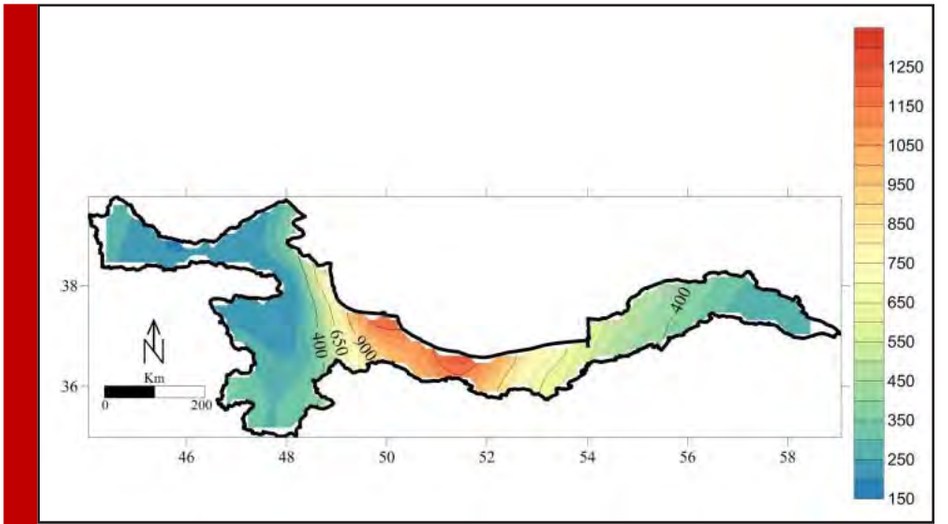

FIGURE 10. Map of simulated precipitation (mm) for the future period (2046-2065).

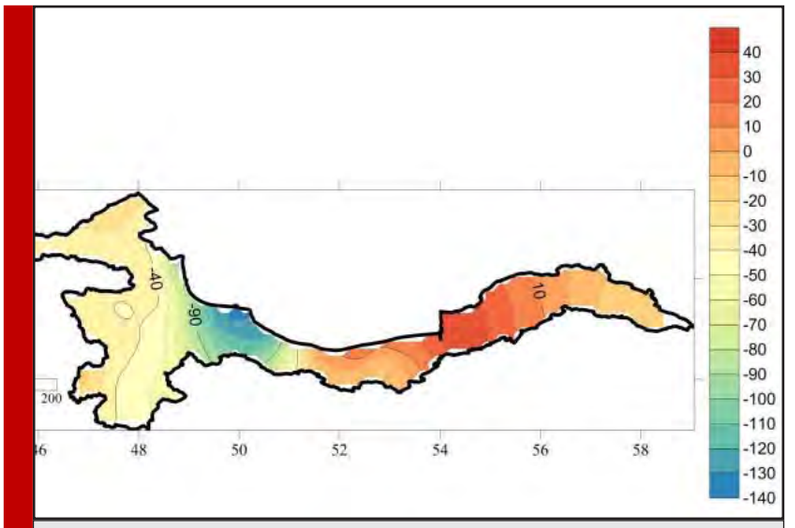

FIGURE 11. Map of difference of precipitation (future-base)

Table 1. Change of the observed and simulated climatic variables across the Basin.

	Min temperature (°C)	Max temperature (°C)	Precipitation (mm)
Mean of observed (1992-2015)	5.4	17.2	555.5
Mean of simulated (2046-2065)	9.2	20.4	518.4
Difference (future – base)	3.8	3.2	-37.1

CONCLUSION

In this investigation the climatic variables, including min, max temperature and precipitation were applied from 21 synoptic stations across the Khazar Basin and were then downscaled using LARS- WG weather generator for the future period (2046-2065). The findings of this study revealed that over the future period the min and max temperature will increase across the Basin and the rate of the increase is 3.8 and 3.2 °C respectively. In some parts of the Basin in the north-west, the rate of increase for min temperature is up to 6.8 °C while for max temperature it is up to 4.5 °C. The analyses also showed that in general precipitation will decrease for the future period with the average amount of 37.1 mm, but there are some limited areas in the Basin which will experience a little increase for the precipitation. From the obtained results of this study it can be concluded that the Basin will be highly affected by climate change in the future which can have significant impacts on the ecosystem. Therefore serious adaptive steps must be taken to mitigate the adverse effects of climate change because the agriculture in this Basin is mostly rain fed and temperature increase along with precipitation decrease can deteriorate the climatic conditions considerably.

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CONFLICT OF INTERESTS

None declared

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Antibacterial activity of ZrO_2 against metallo beta-lactamase and biofilm producing carcinogenic *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is frequent pathogen associated with hospital acquired infections exhibiting intrinsic resistance to numerous antibiotics. A total of 80 isolates of Pseudomonas spp. were isolated from blood cancer patient from three tertiary hospitals in and around Tirupur and Erode (Dt). Metallo beta-lactamase and biofilm production is the most worrisome resistant mechanisms observed in *P. aeruginosa*. Emergence of antimicrobial resistance by pathogenic bacteria is a major health problem in recent years. The biofilm and Metallo beta-lactamase production was tested by combined disc test and tissue culture plate method. Electrophoretic analysis of the plasmid DNA prepared was carried out by agarose gel electrophoresis on 0.7%. Nineteen carcinogenic isolates which was showed more than 50% resistance against tested antibiotics were selected for plasmid isolation. Recurrently, ZrO_2 nanoparticles comprises of well-known inhibitory and bactericidal effects. The nanoparticles were obtained and tested against 10 Metallo beta-lactamase and biofilm producing carcinogenic isolates. The nanoparticles showed appreciable activity at all tested concentrations (0.2, 0.4 and 0.6 mg/ml). Thus, it is concluded that the present study designed to determine the efficacy of ZrO_2 may serve as a promising antibacterial agent against cancer causing *Pseudomonas aeruginosa*.

INTRODUCTION

Pseudomonas aeruginosa is one of the most important opportunistic bacteria, causing a wide variety of infections especially in immune compromised hosts such as burn patients, patients suffering from respiratory diseases like cystic fibrosis, and cancer chemotherapy patients (Govan and Deretic, 1996). It is gram negative short rod belong to family Pseudomonacaeae. Infections with *P. aeruginosa* is occupy very important position as of greatest concern in critically ill and immune compromised patients who have been hospitalized for extended

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periods of time and have received broad-spectrum antimicrobial therapy or cancer chemotherapy (Pollack, 2000, Ae Mftah *et al.,* 2015).

The pathogenic cancer causing *Pseudo* bacterial life includes stages where the cells are associated and form a biofilm on a surface (e.g. Costerton et al., 1995). The formation of these surface communities and their inherent resistance to antimicrobial agents are the cause of many persistent and chronic infections (Costerton *et al*., 1999).

Nowadays the *P. aeruginosa* presents a serious therapeutic challenge for treatment of both community-acquired and nosocomial infections, and selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome (Mavroidi et al., 2005). Unfortunately, selection of the most appropriate antibiotic is complicated by the ability of *P. aeruginosa* to develop resistance to multiple classes of antibacterial agents, even during the course of treating an infection. Further acquired resistance is also reported by the production of plasmid mediated AmpC beta (β)–lactamase, Extended Spectrum Beta (β)–Lactamase (ESBL) and metallo beta (β)–lactamase (MBL) enzymes.

Currently, the nanoparticles are increasingly recognized for their utility in biological applications including nano medicine (mariappan et al., 2011). The ongoing worldwide nanotechnology revolution is predicted to impact several areas of biomedical research and other science and engineering applications. Nanoparticles-assisted drug delivery, cell imaging, and cancer therapy are important biomedical applications of nanotechnology (Hua Wang et al., 2011).

Despite their potential biomedical applications, very few studies have reported on the role of zirconia nanoparticles as anticancer materials. As far as the authors know, this is the first report of the in vitro anticancer effect of sulphated zirconia nanoparticles against three cancer cell lines. Specifically, the toxicity of sulphated zirconia nanoparticles against human colon cancer HT29, human breast cancer MCF-7 and human liver cancer HepG2 cell lines was assessed, showing promising results (Ae Mftah *et al.,* 2015).

Moreover, it is reported here that these novel nanoparticles hold promise not just for anticancer applications but also for anti-infection applications. The steady increase in the antimicrobial resistance of microorganisms represents a great public health concern. This requires the search for new unconventional antimicrobial agents. Nanotechnology provides promising materials to fight infectious diseases such as nanoparticles with antimicrobial activities. So hence the present study has made an attempt to point out the antimicrobial properties of the ZrO_2 nanoparticles were determined using the agar diffusion method against cancer causing *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

A total of 100 clinical cancer blood samples were collected from the jugular vein with a sterile syringe of different age group of blood cancer patients in different clinics and PHC (Primary Health Centre) from in and around Tirupur and Erode District. 80 isolates of *Pseudomonas aeruginosa* were isolated from the samples. Colony morphology was reported as non-mucoid or mucoid and the isolated colonies were subjected to standard biochemical tests and 16S r-DNA gene sequencing for identification of *Pseudomonas aeruginosa*. Finally all isolates were kept at – 20°C in media containing 8% dimethyl sulfoxide (DMSO) until use.

The qualitative and quantitative analyzes of the biofilm produced were performed of clinical isolates of *P. aeruginosa* according to the protocol described by (Xicohtencatl - Cortes et al. 2015). Clinical strains of *P. aeruginosa* were incubated for growth in trypticase-soy broth (TSB) at 37°C for 24 h. For biofilm formation, 24-well plates containing 1 ml of TSB were inoculated with 50 ml (1.5 x 108 bacteria/ml) of a bacterial suspension of each of the *P. aeruginosa* strains and incubated at 37°C for 24 h. Biofilms were washed with phosphate buffer solution (PBS) (*p*H 7.4) and fixed with 2% formalin at 4°C overnight. Subsequently, the fixative solution was removed with PBS and the films were stained with 1 ml of 1% crystal violet for 15 min. Excess crystal violet was removed and 1 ml of methanol at 70% was added for quantification of the biofilm to an optical density of 600 nm.All imipenem resistant isolates were tested for MBL by Imipenem- EDTA combined disc test (CDT).

The Imipenem- EDTA combined disc test (CDT) was performed as described by the method of Yong et al. (2002). The test organisms were inoculated on the plates with seeded Mueller Hinton agar as recommended by the CLSI. A 0.5 M EDTA solution was prepared by dissolving 18.61g of EDTA in 100 ml of distilled water and adjusting its *p*H 8.0. The Mixture was sterilized by autoclaving. After two Imipenem (10µg) discs were placed on the surface of agar plate, add finally 4µl of 0.5 M concentration of EDTA solution. Finally, the inhibition zones of imipenem- EDTA discs were compared after 16 to 18 h of incubation in air at 37°C. In the combined disc test, if the increase in inhibition zone with imipenem- EDTA disc was ≥7 mm it was considered as Metallo Beta Lactamase positive (Lee et al. 2001).

The blood cancer causing *P. aeruginosa* was treated with various different concentrations (0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL & 100µg/mL) of ZrO_2 nanoparticles no significant difference in the growth curve. The growth experiment under the influence of Zirconium nanoparticles thus reveals the non-toxic nature of the Zirconium nanoparticles in the bacterial system. In the

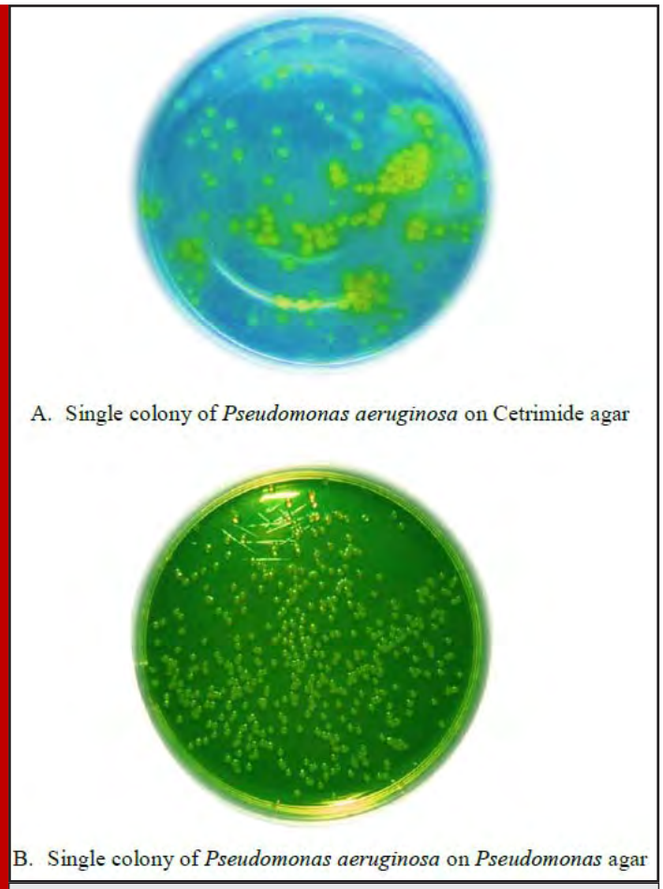
present study plasmid DNA was done by boiling preparation method (Holmes & Quigley, 1981; modified by Riggs & McLachlan, 1986).

Antimicrobial activity of protease was carried out by using well diffusion method. The *Pseudomonas aeruginosa* was inoculated in LB broth and incubated for 24 hrs at 37°C. The turbidity of the broth was adjusted at 0.5 (optical density) using Spectrophotometer. The bacterial culture was inoculated on MHA plates using sterilized cotton swab. Allow it to dry for 2 – 5 min. Well was made using sterile cork borer. Various different concentrations (0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL & 100µg/mL) of different µl of ZrO_2 nanoparticles (50µl, 100µl and 150µl) was impregnated in to the well. The plates were swabbed with *Pseudomonas aeruginosa*. The plates were examined to measure the zone of inhibition around the well.

RESULTS AND DISCUSSION

The carcinogenic disease is caused by external factors, such as tobacco, infectious organisms, and an unhealthy diet, and internal factors, such as inherited genetic mutations, hormones, and immune conditions. These factors may act together or in sequence to cause cancer. Currently, emerging and reemerging infectious disease are a major problem in public health and global economics. They are caused by different type of infection such as drug resistant infection, mostly involving bacteria, and many emerging pathogen is increasing significantly over time because they are becoming progressively more resistant to conventional antibiotic compounds. For example, *Pseudomonas aeruginosa* has been reported as an opportunistic pathogen and one of the most important opportunistic bacteria, causing blood cancer by the intrinsic resistance to many antimicrobial agents. So, the need for the development of new antibiotics to counter drug resistance in bacterial pathogens has been stressed by various researchers worldwide.

Totally 100 carcinogenic blood samples were collected from the jugular vein with a sterile syringe of different age group of blood cancer patients in different clinics and PHC (Primary Health Centre) from in and around



A. Single colony of *Pseudomonas aeruginosa* on Cetrimide agar

B. Single colony of *Pseudomonas aeruginosa* on *Pseudomonas* agar

PLATE 1. Isolated of colonies *Pseudomonas aeruginosa from Blood Cancer*

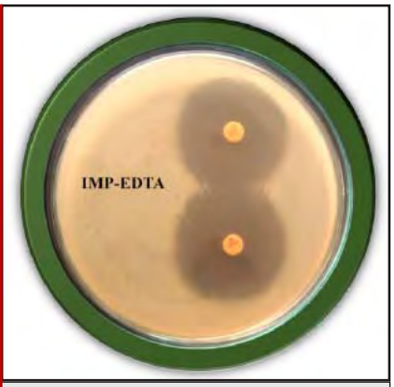


PLATE 2. Detection of Metallo β-Lactamase by Imipenam EDTA Combined Disc Test

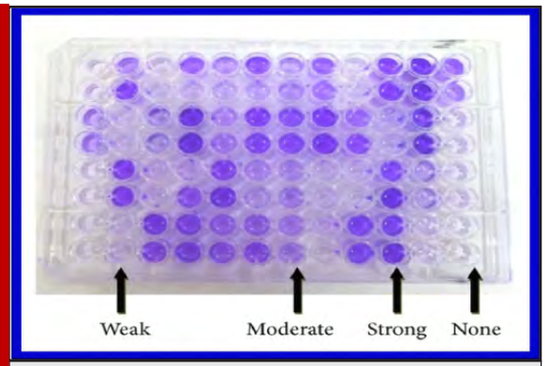


PLATE 3. Biofilm production by carcinogenic pathogen *Pseudomonas aeruginosa*

Tirupur and Erode District. 80 isolates of *Pseudomonas aeruginosa* were isolated from the samples. The cancer causing leading pathogen *Pseudomonas aeruginosa* strains were confirmed by comparing the results with standard biochemical test and 16S-rDNA gene sequence.

In the study 28.75% of blood cancer causing *Pseudomonas aeruginosa* isolates was found to be MBL producers. These isolates exhibited a ≥ 7 mm zone enhanced for Imipenem and EDTA combined than the Imipenem disks alone. All the MBL producing isolates were multi drug resistant, most of which showed resistance to more than twelve antibiotics out of the twenty antibiotics tested. Twenty three isolates: PARS01, PARS02 PARS03, PARS05, PARS06, PARS21, PARS41, PARS43, PARS44, PARS47, PARS51, PARS56, PARS58, PARS60, PARS61, PARS62, PARS63, PARS64, PARS74, PARS75, PARS74, PARS75, PARS77, PARS78 and PARS80 which was showed more than above 50% resistance used for Metallo Beta Lactamase production by Imipenem (IMP)-EDTA Combined Disk test of (Yong et al. 2002). The maximum zone of inhibition (mm) produced by the strain No. PARS21 and PARS 77 which showed 40 mm followed by minimum amount of zone of inhibition produced by the strain No. PARS56 and PARS64 which showed 30mm (Plate: 8; Table: 16). Electrophoretic analysis of the plasmid DNA prepared was carried out by agarose gel electrophoresis on 0.7%. Nineteen carcinogenic isolates (PARS01, PARS02, PARS03, PARS05, PARS06, PARS21, PARS41, PARS43, PARS44, PARS47, PARS51, PARS56, PARS58, PARS60, PARS61, PARS63, PARS64 and PARS80) which was showed more than 50% resistance against tested antibiotics were selected for plasmid isolation.

The antibacterial activity of ZrO_2 nanoparticles was studied by agar well diffusion method. Three different concentrations (0.2%, 0.4% and 0.6%) were used in this assay against leukemia (blood cancer) causing *Pseudomonas aeruginosa*. The maximum zone of inhibition 18mm, 19mm and 22mm was observed in strain no. PARS47 followed by the minimum zone of inhibition 15mm, 16mm and 17mm was observed in strain no. PARS64 and PARS56 at 150µl concentration of different µg- 50, 100 and 150 of ZrO_2. Mahrukh Khattak *et al.*, (2013) determined the frequency of *Pseudomonas aeruginosa* in middle and outer ear and to check the antibiotic susceptibility pattern of commonly used antibiotics. The pathogen was highly sensitive to Gentamycin 30 (71.4%), followed by Aztreonam 28(66.6%), Cefixime 22(52.3%), Imipenem 18(42.8%), amikacin 15(35.5%) and Ciprofloxacin 12(28.5%). Females were more susceptible to *Pseudomonas* infections. Isolates of *Pseudomonas*

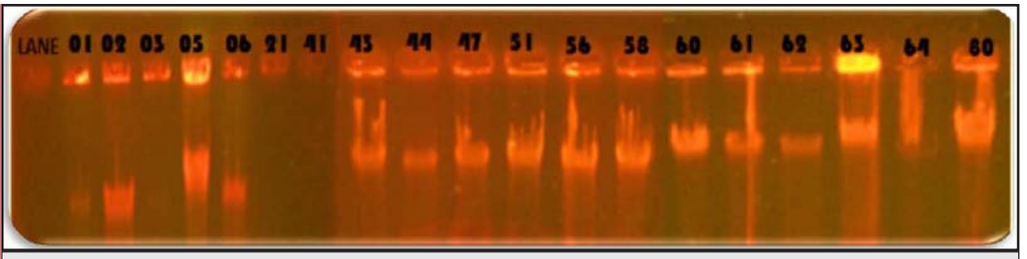


PLATE 4. Isolation of plasmid from *Pseudomonas aeruginosa*

PLATE 5. Antibacterial activity of ZrO_2 against Metallo beta-lactamase and biofilm producing carcinogenic *Pseudomonas aeruginosa*

aeruginosa from samples showed highest sensitivity (71.4%) to Gentamycin while maximum resistance was showed to Ciprofloxacin (47.6%). In this research, similar results were found in all the *Pseudomonas aeruginosa* in blood cancer origin were more highly sensitive to Imipenem (97.5%).

Resistance to imipenem has been found to be independent of β-lactamase production and in *P. aeruginosa* has been attributed to diminished expression of certain outer membrane proteins (Buscher *et al.,* 2000). More than 80% of isolates in this study were sensitive to imipenem (97.5%). Compared with results of a study conducted at the Lagos University Teaching Hospital (LUTH) in which 12.5% were resistant to imipenem, in this study only 5.4% *Pseudomonas* strains were resistant. Imipenem is a drug that is not readily available in our environment and its cost is also prohibitive.

Plasmid analysis of the multi-resistant strains showed that 18 of the *Pseudomonas* strains harbored plasmids, eleven of which had similar plasmid band patterns of 1-7 plasmid bands having low to intermediate molecular weights. Plasmid prevalence was higher in the strains from blood cancer. Acquisition of mobile genetic elements is known to be the main mechanism for short term accumulation of resistance determinants in bacterial genomes (Liu *et al.,* 2000).Our finding that 18(78.75%) out of the 46 *Pseudomonas* isolates contained plasmids with seven different plasmid profiles, coincides with that reported by Tsakris et al., (1992) who found that 10 isolates harbored plasmids out of 35 multi resistant pseudomonas strains, with 6 different plasmid profiles. In another study, Poh *et al.,* (1988) detected 11 different plasmid profiles in *Ps. aeruginosa* isolates.

Biofilm producing organisms are responsible for many recalcitrant infections and are notoriously difficult to eradicate. They exhibit resistance to antibiotics by various methods like restricted penetration of antibiotic into biofilms, decreased growth rate and expression of resistance genes (Hachem *et al.,* 2007). There are various methods for biofilm detection (Poole, 2004). In the present study the number of isolates showing strong biofilm producers were 41(64%) and moderate biofilm producers were 39(36%) by Tissue culture plate method similar to the study done by Afreenish Hassen *et al.,* (2011). In their study noted that out of 110 isolates from different clinical samples tested for biofilm production, the number of biofilm producers identified by Tissue culture plate method (TCP) was 70(64.7%) and non or weak biofilm producers were 40(36.3%). The recent study has performed the Tissue culture plate method by addition of 1% glucose in trypticase soy broth. Addition of sugar helps in biofilm formation16. This was also reported by studies conducted by Mathur *et al.,* (2006).

Metallo-beta-lactamase enzyme is an emerging threat and cause of concern for physician. The metal ion active sites appear to decrease their susceptibility to beta lactamase inhibitors and enable them to hydrolyze broad spectrum including carbapenems. The Metallobeta-lactamase are plasmid mediated, so the resistance can be spread among hospital pathogen and will cause problems in treating infections (Mehul Chaudhari *et al.,* 2011). In present study, attempt was made to detect Metallobeta- lactamase producing *Pseudomonas aeruginosa*. Of 80 isolates of *Pseudomonas aeruginosa*, 23 (2.5%) were resistance to imipenem. All 23 isolates were found to be MBL producers. Of 23 isolates of MBL, 23 (28.75%) were isolated from blood cancer patient. The prevalence of detect Metallo-beta-lactamase producing *Pseudomonas aeruginosa* in our setup if 2.5%.

Ae Mftah *et al.,* (2015) studied the sulphated zirconia nanoparticles showed high antimicrobial activity against both Gram-positive and Gram-negative bacteria. It was found that the nanoparticles showed the highest activity against *Pseudomonas aeruginosa* and methicillin-resistant *S. aureus*, followed by *Bacillus subtili*s and *Salmonella choleraesuis*. The first time that the exposure of cancer cells to sulphated zirconia nanoparticles (3.9–1,000 µg/mL for 24 hours) resulted in a dose-dependent inhibition of cell growth, as determined by (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays. Similar promising results were observed for reducing bacteria functions. In this manner, this study demonstrated that sulphated zirconia nanoparticles with bronsted acidic sites should be further studied for a wide range of anticancer and antibacterial applications. Followed by these present study similar results of antimicrobial ability of ZrO2 at different concentration (0.2%, 0.4% and 0.6%) used against blood cancer causing *Pseudomonas aeruginosa*. Zirconium oxide was

used and it shows prominent anti carcinogenic activity against test pathogen.

CONCLUSION

The study also highlights that MβL incidence is increasing in our region. The metallo beta-lactamase and biofilm production is the most worrisome resistant mechanisms observed in *P. aeruginosa*. Emergence of antimicrobial resistance by pathogenic bacteria is a major health problem in recent years. The resistance may spread rapidly to various species of Gram-negative bacilli; therefore, to prevent the further spread of MβL producers, it is essential to rapidly detect MβL-positive isolates to aid infection control. Recurrently, tiny nanoparticles, far smaller than the width of a human hair, might help the body's own immune system fight tumors. Moreover, it is reported here that these novel metallo nanoparticles (ZrO_2) comprises of well-known inhibitory and bactericidal effects against cancer causing *Pseudomonas aeruginosa*.

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Physicochemical and fungal diversity analysis of two different sources of polluted water of Cachar District Assam

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ABSTRACT

Analysis of physicochemical and fungal species from two different sources of water pollution, as Paper Mill Effluents (PME) of Hindustan Paper Corporation and City Domestic Sewage (DS) of Cachar district, Assam was carried out during pre and post monsoon season (February, 2016 and May, 2016). Water samples were collected and analyzed for standard physicochemical parameters where pH, turbidity, electrical conductivity, total hardness were found within the limits prescribed by WHO and ISI where as total alkalinity, Total Dissolved Solids (TDS), nitrate and Phosphate content were found to be exceeding the permissible limits for both PME and DS. *Aspergillus niger, A. flavus.* and *Penicillium citrinum* were among the most commonly encountered species of fungi from both the sites. Highly significant microbial load was observed in domestic sewage (cfu/ml =22.0x103, P<0.001) during the post monsoon season (May, 2016).

ABBREVIATIONS: PME: PAPER MILL EFFLUENT; DS: DOMESTIC SEWAGE

KEY WORDS: PAPER MILL EFFLUENTS, DOMESTIC SEWAGE, FUNGAL SPECIES, PHYSICO CHEMICAL PARAMETERS

INTRODUCTION

Water is the basic need of life, a precious gift of nature to man and all the living creatures of this universe. So the quality of water is of vital concern as it is directly linked with human welfare. Only 3% of the water in the universe is fresh and among the fresh waters, only around 5% or 0.15% of the total water are available for use. The total water resource available in India is 1850 km^3, which is approximately 4% of the worlds fresh water resources (EPA-PWD, 2001) . But very fast it is becoming a scare commodity in many parts of the universe.

The water quality monitoring results obtained during 1995 to 2006 indicate that the organic and bacterial

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contamination are continued to be critical in water bodies. This is mainly due to discharge of domestic wastewater mostly in untreated form from the urban centers of the country and at the same time the receiving water bodies also do not have adequate water for dilution. Therefore, the oxygen demand and bacterial pollution is increasing day by day which is mainly responsible for water borne diseases.

The total surface water resource of Assam State is estimated at about 600 billion cubic meters, where the annual replenishable groundwater resource of the State has been estimated as 27.23 billion cubic meters. But prolonged discharge of industrial effluents, domestic sewage and solid waste dump causes pollution in the water resources which leads to enormous health problems. The rapid growth of industrial resources has further affected water quality due to overexploitation and improper waste disposal practices. Hence there is always a need for concern over the protection and management of water resources .considering the above aspects of contamination ,the present study was undertaken to investigate the level of water pollution and at the same time to measure the microbial contamination due to industrial and domestic exposure in the country rivers.

MATERIALS AND METHODS

SAMPLING SITE AND PREPARATION OF WATER SAMPLES

The present study was carried out during the pre and post monsoon season (February, 2016 and May, 2016). Raw paper mill effluent sample was collected from the outlet pipes in the local river Barak. For comparative analytical study domestic sewage was collected from Silchar, Municipal drainage system at Tarapur area, where all the debris and discharges of the whole locality have been found to be discharged. Both the effluent and sewage samples were collected in plastic gallons, pH was measured and stored at -20°C to prevent further microbial growth.

PHYSICO-CHEMICAL PARAMETERS AND MICROBIAL DIVERSITY STUDY

The paper mill effluent and domestic sewage were analyzed for a number of standard physico-chemical properties, including Color, temperature, pH, turbidity, Dissolved Oxygen (DO), Electrical Conductivity (EC), Total alkalinity (TA), Total Dissolved Solids (TDS), Total Hardness (TH), $FCO_{2,}$ Nitrate and Phosphate content were analyzed followed by the standard methods (USEPA,1996; APHA,1998).

For the isolation of fungi from both the effluent and sewage samples , serial dilution plate technique was used .1 ml of final dilution of sample was inoculated on Czapek Dox Agar medium. In sterile Petri dishes Martin's Rose Bengal was added as a bacteriostatic agent according to Smith and Dawson (1944). The tubes of colony forming units (cfu) was determined after incubating the inoculated plates in 3 replicates at 25°C (±2°C) for 4-5 days, thereafter fungal species were identified using the keys as described by Gilman (1957); Raper and Fennel (1965).

STATISTICAL ANALYSIS

Results were presented as Mean±SE followed by microbial diversity studies where mean value was calculated from three individual readings of a particular set. ANOVA was performed to determine the level of significance of microbial diversity studies. ANOVA was done using graph pad PRISM (Graph pad Inc., san Diego, CA, USA). Percentage occurrence used in this study include ,

$$\text{Percentage occurrence of species} = \frac{\text{Occurrence of Species A}}{\text{Occurrence of all species}} \times 100$$

RESULTS AND DISCUSSION

The result of physicochemical parameters performed from both paper mill effluent and domestic sewage are presented in Table 1 and 2.

PH

pH is used to express the intensity of acid or alkaline condition of a water sample. In our case pH value ranged from 4.78-6.74, found within the permissible limits. Highly acidic pH was recorded in DS in both the seasons specially during the month of May. Water samples with low pH(<6.0) attributes to the discharge of acid contents into these effluents by agricultural or domestic activities. A decrease in pH may be caused by the increase in the amount of organic carbon, total carbonates available in the sewage resources. Though pH has no direct effect on human health still the different kinds of biochemical reactions taking place within human body are sensitive to variation of pH.

TURBIDITY

Turbidity is due to colloidal and fine dispersions in water resources. The turbidity value varied between 2.5-3.2 and found within the permissible limit of WHO and ISI.

DISSOLVED OXYGEN (DO)

Dissolved Oxygen is an important parameter for water quality assessment which reflects the biological pro-

Table 1. Physico chemical characteristics of PME and DS in the month of February, 2016.

Parameters	Paper Mill Effluent (PME)	Domestic Sewage (DS)	WHO Standards	ISI Standards
Color	Pale brown	Dark brown	-	-
Temperature	28	24	-	-
pH	6.16	4.78	6.5-8.5	6.5-8.5
Turbidity	2.5	2.8	5.0	10
Dissolved Oxygen (DO)	23.24	4.44	-	5.0
Electrical conductivity (EC)	0.9	1.9	1400	
Total Alkalinity	460	355	120	200
Total Hardness	214.67	138.67	500	300
FCO_2	7.34	14	-	-
Total Dissolved Solids (TDS)	665	3695	1000	500
Nitrate	7.942	8.414	5	45
Phosphate	2.192	3.778	0.1	-

Table 2 . Physico chemical characteristics of PME and DS in the month of May, 2016.

Parameters	Paper Mill Effluent (PME)	Domestic Sewage (DS)	WHO Standards	ISI Standards
Color	Pale brown	Dark brown	-	-
Temperature	26	23	-	-
pH	6.74	5.12	6.5-8.5	6.5-8.5
Turbidity	2.7	3.2	5.0	10
Dissolved Oxygen (DO)	17.96	3	-	5.0
Electrical conductivity (EC)	1.2	2.1	1400	
Total Alkalinity	417	323	120	200
Total Hardness	298.67	202.66	500	300
FCO_2	14.34	24.67	-	-
Total Dissolved Solids (TDS)	617	3432	1000	500
Nitrate	8.123	9.235	5	45
Phosphate	2.431	4.537	0.1	-

All parameters are in mg/L except Temperature, pH, Turbidity, Electrical conductivity. Temperature in °C, Turbidity in NTU, Electrical conductivity in micromho /cm

Table 3. Result of microbial (fungal) diversity isolated from PME and DS (February, 2016 and May, 2016).

Treatment Groups	cfu /ml (x 103)	
	February, 2016	May, 2016
Paper Mill Effluent (PME)(%)	4.67±0.17	5.67±0.45
10	9.34a±0.45	10.34b±1.2
25	10.67b±0.45	19.34a,b±0.75
50		
Domestic Sewage (DS) (%)	7.34±0.34	8.0±0.78
10	13.67c,b±0.45	12.67b±1.047
25	15.67c,b,a±0.75	22.0b,c,a±1.95
50		

Where, P < 0.05 = a, P < 0.01 = b, P < 0.001 = c

cesses taking place in water bodies. The DO value indicates the degree of pollution in water .In our study the DO content was found minimum during the month of May, which may be because the increased algal productivity due to increased rate of photosynthesis (Rajkumar *et al*, 2004).

ELECTRICAL CONDUCTIVITY (EC)

Electrical conductivity is a measure of water capacity to convey electrical current. It signifies the amount of total dissolved salts. EC value was found within the range of 0.9-2.1 micromho /cm where DS showed higher EC value in both the seasons indicating the presence of high amount of dissolved inorganic substances in the ionized

Table 4. Occurrence of fungi isolated from PME and DS (February, 2016 and May, 2016)

Fungal Species Identified	Water Samples											
	Paper Mill Effluent (PME)						Domestic Sewage (DS)					
	10%		25%		50%		10%		25%		50%	
	Feb	May	Feb	May	Feb	May	Feb	May	Feb	May	Feb	May
A. flavus	42.85	47.05	50	38.7	50	39.65	27.27	0	31.7	0	31.91	0
A. niger	0	41.17	0	48.38	0	44.82	45.45	44.45	39.02	50	40.42	56.06
Penicilliumcitrinum	28.57	11.76	21.42	12.9	25	15.51	9.09	0	9.75	0	10.63	0
A. fumigatus	0	0	0	0	0	0	18.18	51.85	19.51	42.1	17.02	31.81
Mucor sp.	14.28	0	17.85	0	15.625	0	0	3.7	0	7.89	0	6.06
Rhizopus sp.	14.28	0	14.28	0	9.375	0	0	0	0	0	0	0

form in domestic sewage as the conductance of water increases with salts. Higher the concentration of electrolyte in water the more is its electrical conductance.

TOTAL ALKALINITY

The alkalinity of water is caused mainly due to OH, CO_3, HCO_3 ions, borates, phosphates and organic acids. It is an estimation of the ability of water to resist change in pH upon addition of acid. In the present study, alkalinity was found beyond the permissible limits of both WHO and ISI standards. Maximum alkalinity may be due to low temperature bringing down the rate of decomposition of salts to a minimum thus by increasing alkalinity.

TOTAL HARDNESS

Water hardness is a measure of capacity of water to precipitate soap. It is defined as the sum of Calcium and Magnesium concentration, both expressed as Calcium carbonate. In the present investigation, the Total Hardness value (138.67-298.67 mg/L) ranged within the permissible limits with maximum hardness during summer (May), which might be due to reduced inflow of water and rate of evaporation. The FCO_2 value was recorded minimum in PME specially in February, 2010 which may be due to less suspended microbial load on suspended particles and algal masses (Michael, 1984).

TOTAL DISSOLVED SOLIDS (TDS)

TDS content was found within the range of 617-3695 mg/L where DS showed higher TDS content specially in pre monsoon season which exceeded the maximum permissible limit of ISI Standards indicating the enormous storage of different types of salts such as carbonates, bicarbonates, chlorides, phosphates, nitrates, magnesium calcium, sodium, potassium, manganese and organic matters.

Nitrate and Phosphate content were found at higher concentrations in domestic sewage specially in the summer season (May, 2010) where Nitrate and Phosphate level for both the effluents exceeded the permissible limit of WHO Standards. Higher Phosphate concentration may be because of the discharge of domestic wastes, soaps, detergents, fertilizers and human activities.

All together six species of fungi belonging to four genera were isolated and identified from the water samples. The majority of fungi were encountered rarely. Of the genus *Aspergillus* the best represented species were *Aspergillus niger, A. fumigatus A. flavus*. and *Penicillium citrinum* which were isolated from PME as well as from DS. The fungi isolated from different samples of Barak River were likely to be originated from soil or entered the water with plant remains. The isolated fungi from river Barak belong mainly to the category of transient accidental microorganisms, according to ecological classification of aquatic heterotrophic microorganisms (Park, 1972b). Transient and accidental microorganisms can develop sporadic activity and soil fungi may participate in microbiological processes in water bodies (Park, 1972b). Among the fungal population *Aspergillus* was represented by highest number of species. This is in accordance with the statement of Barron (1968) that *Aspergillus* is biologically one of the most successful of all fungi and is expected to occur on all sorts of organic debris. Carlie and Watkinson (1997) in their study observed that *Aspergillus* and *Penicillium* sp have cellulolytic enzymes and wood degrading capability. Cellulolytic filamentous fungi have the ability to penetrate cellulose substrate through hyphal extensions. cfu rate was found to be highly significant in DS ($22.0x10^3$) especially in summer followed by. ANOVA. Low cfu rate in February may be due to cold climatic condition inhibiting fungal growth. Maximum fungal diversity was recorded in domestic sewage in comparison to PME. Higher occurrence of *A. flavus* and *Penicillium citrinum*. was noted in PME where as *A. niger* and *A. fumigatus* were among the mostly encountered species in DS in both the seasons.

CONCLUSION

As the river water is being purified by the municipal corporations before public consumption, so human being

have least possibilities to get effected as the effluents and sewages get diluted in the river and after purification possibilities to develop water borne disease becomes negligible. But aquatic flora and fauna are directly getting exposed to the water of river Barak , might be tremendously effected by the exposure of this polluted water specially in the form of microbial contamination as no precautions have been undertaken yet for the safety of aquatic ecosystem including the aquatic plants and organisms specially fishes. So, proper measures have to be undertaken for the maintenance of water quality in Barak river, so that the water quality and aquatic life including the aquatic flora and fauna can be protected from microbial contamination and water pollution as well.

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Comparison of multiplex ligation-dependent probe amplification and qPCR for screening PAX5 gene detection in acute lymphoblastic leukemia

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ABSTRACT

Despite the advances in diagnosis of leukemia, still we need a rapid and cost-benefit screening method in patients with acute lymphoblastic leukemia (ALL). The aim of the present study is to evaluate the ability of Multiplex Ligation-dependent Probe Amplification (MLPA) method in screening patients with ALL. 45 patients with ALL were selected. DNA were extracted, then PAX5 gene copy number abnormalities (CNAs) were studied by adopting MLPA and QPCR methods. To prove the results of two methods, PCR product from three samples were sent for sequencing. From 45 patients with B-ALL, 11 (24%) patients, showed CNAs after applying MLPA method. From among all mutations, 22 samples (29%) were seen in PAX5 gene. We used Sanger sequencing as a gold standard method to compare the two methods. After sequencing, we were submitted 2 genes in Gene Bank by accession numbers (ID) KX608846 and KX707789 in exon 10 of PAX5. MLPA is a rapid and valid method for screening of genes mutation and can be used in these laboratories as routine method especially in low-income countries.

KEY WORDS: ACUTE LYMPHOBLASTIC LEUKEMIA, COPY NUMBER ABNORMALITY, MLPA, QPCR

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INTRODUCTION

Today, B-cell acute lymphoblastic leukemia (B-ALL) is the most common childhood malignancy, especially in children below five years of age. Today, we have extensive knowledge about leukemia like other cancers. In the last two decades, molecular methods have provided the physicians and researchers with a new vision towards the role of molecular factors in pathogenesis of diseases. Alternative expression leads to the abnormal proliferation and differentiation of lymphoid ancestors (Fazio, Biondi, & Cazzaniga, 2011; O'neil & Look, 2007 Seiter et al., 2014).

Paired Box5 (PAX5) is an important transcription factor located on chromosome 9p13 and includes 10 exons. PAX5 is essential factor for the development of pro-B cell to mature B-cell(Fazio et al., 2011). Up to now, nine PAX transcription factors have been demonstrated, although, PAX5 is the only PAX protein which is expressed in the hematopoietic system (Busslinger, 2004; Cobaleda, Schebesta, Delogu, & Busslinger, 2007; Matthias & Rolink, 2005). The improvement of diagnostic and treatment protocols by using risk-adapted methods have enhanced cure rate up to 80%(Carter et al., 2001).

Therefore, the identification of biomarkers can be a great help for the patients and the health-care system to reduce the health-care costs and side effects of chemotherapy. Screening is highly pivotal in the developing countries where the financial resources and drugs are limited. Childhood cancer rates has been rising slightly in the past few decades (Fathi et al., 2015).

In Iran, leukemia has an increasing trend and leaves a heavy burden on the whole society,(Fazeli et al., 2013). Thus, more attention needs to be paid to screening, early diagnosis, and effective treatment in order to increase the survival rate for children's cancer (Mousavi et al., 2008).

In this study, we carried out Multiplex Ligation-dependent Probe Amplification (MLPA) analysis on 45 ALL patients' samples to determine the copy number abnormality (CNA) of PAX5 gene. MLPA is a technique capable of showing variations in the copy number of several human genes.Due to this capability, MLPA is mostly used in the molecular diagnosis of several genetic diseases whose pathogenesis is related to the presence of deletions or duplications of specific genes,(Schouten et al., 2002; Stuppia, Antonucci, Palka, & Gatta, 2012). Also, we adopted the real-time PCR method for comparing the results of two methods.

MATERIALS AND METHODS

58 Bone marrow(BM) or/and peripheral blood (PB) samples were taken at the time of diagnosis of patients with B-ALL, who were presented to the Children's Hospital of Tabriz University of Medical Sciences, between 2014 and 2016. The selection criteria included having at least 20% blast cells, and being between 1-14 years of age at the time of diagnosis. The exclusion criteria were the samples from foreign patients and DNA with insufficient quality. Six samples were excluded due to failure of MLPA, Five samples due to bad quality of DNA and two of them were withdrawn. Additionally, 4 bone marrow and 7 peripheral blood samples from healthy donors were analyzed as control samples. Graphical abstract of diagnosis process in this study are shown in (Figure 1).

The written informed consent was obtained from all the parents and the study protocol was approved by Ethics and Human Rights Committee of Tabriz University of Medical Sciences.

The DNA was extracted from BM or PB samples, using the QIAamp DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. In order to verify the fidelity of DNA concentration and to evaluate its quality, a spectrophotometer,(NanoDrop,Wilmington DE,,USA) and 1.5% agarose electrophoresis gel were used respectively.

Two pairs of primer for exone10 of PAX5 gene were designed using the online program oligo7. The primer sequences are shown in (Table 1). SYBR Green Real-time PCR (BIO-RAD iQ5, Bio-Rad, Hercules, California, USA) was done using a serial dilution of DNA samples including 100, 50, 25, 12.5 and 6.25ng in triplicate repeats. Then, according to the standard curves and by comparing the slope and efficiency of each reaction (Schmittgen, Lee, & Jiang, 2008), 50 ng of DNA were preferable as the best concentration. The analysis was performed in a total volume of 20 µl including 50 ng of DNA, 0.2 mM specific primers and 10 µl of SYBR Green Master mix (Applied Biosystems, Foster City, California, United States) according to the manufacturer's instructions, then ($2^{-\Delta\Delta Ct}$) was calculated by normalizing samples and the relative gene copy numbers were calculated. The

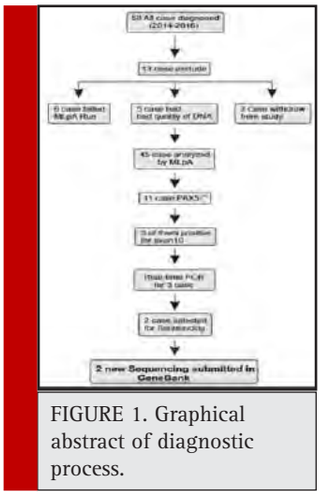

FIGURE 1. Graphical abstract of diagnostic process.

Table 1. The primer sequencing used in QPCR for PAX5 copy number abnormalities.

Gene name	Product length	Tm(C°)	Primer sequence
PAX5 F1	170	64	5-ATCTTAACCTAGGCAGAGCATC-3
PAX5 R1	170	61	5-CAAGAGACACACCATTTGGG-3
PAX5 F2	164	62	5-CTCCTTCTTAGTATCTACGAG-3
PAX5 R2	164	60	5-GAACTCAAAGAAACTGTCTGG-3
GAPDH F	120	60	5 -CGGTGGATCCCTTTAATTG-3-3
GAPDH R	120	58	5 –CAATAAAGGGGATCCACCG-3

copy numbers compared to the reference gene were determined. Following values were considered for result analysis: 1 for normal samples, 0.5 for heterozygous deletions, and 1.5 for heterozygous duplications. Results were analyzed using Microsoft Excel.

The MLPA probe sizes, chromosomal position and sequences and other complete details are available on the Website (www.MRC-Holland.com). The sensitivity of the MLPA kit P335-B2 was assessed previously by Kuiper *et al* (Kuiper et al., 2007) . In each run of MLPA, appropriate numbers of the samples, and three to six control samples were used in same run. All control samples were taken from the children with no acute leukemia or other types of malignancy. In this study, 50 ng of genomic DNA in final volume of 5 µl were used to determine copy number of PAX5 gene by using the SALSA MLPA kit P335-B2 (MRC Holland, The Netherlands). Capillary electrophoresis were run on 3130XL Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) and amplified PCR products were analyzed by Gene Marker version 1.95 (Soft Genetics State College, Pennsylvania, USA). Values over < 0.75 and > 1.35 were considered to be abnormal and values below 0.25 indicated biallelic deletion. Schematic MLPA analysis is shown in (Figure 2).

Normality of data was calculated by Kolmogorov-Smirnov test and was presented by Mean and Standard Deviation. Non-normal variables were incidental as Median (Max & Min). The gene CNAs difference between the ALL and control samples were calculated by independent sample T-test and regression tree for accuracy of MLPA method. P-values less than 0.05 were considered statistically significant. All statistical analyses were done using the Statistical package for the Social Sciences (SPSS), version 16.0 (SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

45 patients with ALL, including 27 (60%) boys with mean age of 5.8±3.4 and 18 (40%) girl with mean age of 6.0 ±2.7 years were studied (p>0.05). Statistically, no significant differences were seen between the mean ages of genders. Demographic data of the participants have been summarized in (Table 2).

We found a wide range of alterations in the PAX5 gene in this study; deletion or duplication in one or more exons. In 2 cases, deletion and duplication were seen in different exons simultaneously. From among all patients, 11 (24%) patients showed 76 CNAs, from which, 22 (29%) were seen in PAX5 gene. The more frequent exons were 6, 7, and 8 (55%); the least one was exon 1 (4%). No significant difference was observed between CNAs and sex, age, National Cancer Institute (NCI) risk group or source of samples (p<0.05). Schematic detected CNAs are shown in (Figure 3).

From among all samples, three samples showed changes in exon 10 by MLPA. All of these samples (3) were validated by real-time PCR. The standard melting curve of real-time PCR is shown in (Figure 1).

The accuracy obtained for MLPA method for detection of CNAs comparisons of real-time PCR was 86%.

To prove the results of two methods, PCR product from these patients was sent to Bioneer for sequencing. The sequencing analyses were done by Chromas software, and the results were compared with the reported gene sequences. We submitted 2 genes in GenBank by accession numbers (ID) KX608846 and KX707789 in exon 10 of PAX5. Schematic representation of each method is shown in (Figure 4).

ALL is a heterogeneous disease with differing in response to chemotherapy. The results of some studies demonstrated that the identification of molecular markers may improve the treatment approaches (Yang et al., 2011). Today, the determination of gene dosage is important for both clinical and research medicine and it is also required for therapeutic, prognostic, or diagnostic goals (Ginsburg & Willard, 2009; Vogelstein & Kinzler, 2004). Due to the growing need for molecular studies, a lot of new molecular markers have been discovered (Mrózek,

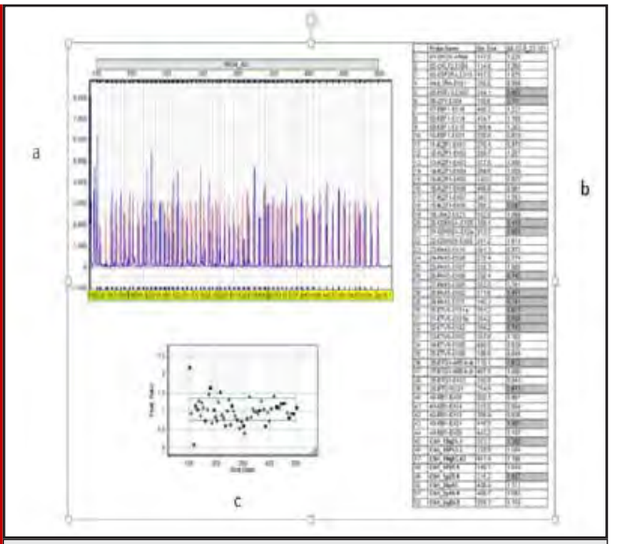

FIGURE 2. MLPA analysis sheet; a: compares peaks between standard and patients probes; b: peaks ratio; c: demonstrated probes ratio in cut-off range (Red peak: mean of whole samples peak, Blue peaks: samples peak, Black peak: reference peak).

Döhner, & Bloomfield, 2007; Thiede et al., 2006). It seems that in the future, patients could be divided into two groups in terms of presence or absence of these biomarkers (Mi et al., 2012).

PAX5 translocations do not influence the prognosis or outcome by themselves but it is in association with other molecular aberrancies in theirs pathway that they influence the prognosis or outcome (Sellner & Taylor, 2004). Similar to our findings, the incidence of PAX5 rearrangements was seen about 30% in the B-cell precursor ALL, in a previous study (Barbosa et al., 2015; Hunger & Mullighan, 2015; C. Schwab et al., 2010; C. J. Schwab et al., 2013). Array-based approaches can provide high resolution data on CNAs, but these methods are restricted by low throughput, high cost, and time consumption. Therefore, the need for a simple, fast and cost-efficient method to screen molecular changes has remained hitherto. (Vermeesch et al., 2007).

The PCR-based multiplex ligation-dependent probe amplification (MLPA) technique is a relative quantification method for gene dosage determination and deletions/duplications mutations in unknown samples which are recognized by comparison to the normal and standard control (Al Zaabi, Fernandez, Sadek, Riddell, & Greer, 2010). Buijs et al. performed genomic profiling using MLPA in 45 cases with (ALL), showing that MLPA is able to detect anomalies similar to PCR method (Buijs, Krijtenburg, & Meijer, 2006).

A similar study was done by Coll-Mulet et al., who carried out MLPA in 50 chronic leukemia patients. Their results illustrated that only cases with a low percentage (<25%) of cells, were not detected by MLPA (Coll-Mulet et al., 2008). Many other studies on different types of leukemia revealed excellent accuracy and specificity of MLPA as compared to QPCR and showed clinical capability of these techniques with different disease(Abdool, Donahue, Wohlgemuth, & Yeh, 2010; Donahue, Abdool, Gaur, Wohlgemuth, & Yeh, 2011; Fabris et al., 2011; Mullighan et al., 2008; C. Schwab et al., 2010; Schwartz & Dunø, 2004).

However, there are still some limitations such as being unable to detect balanced rearrangements, mosaicism, heterogeneity or contamination with normal cells. Like PCR, MLPA reactions are also more sensitive to contaminants (Stuppia et al., 2012; Tavtigian & Le Calvez-Kelm, 2007). According to the manufacturer's instructions of MLPA, it works with only 20 ng of DNA. Besides, the results don't depend on the amount of used sample DNA but, our study showed that 50 ng are required for reliable results.

Moreover, it is recommended to compare different MLPA analyses only by using one DNA extraction method from different sources of samples from each patient, to compare specify the sensitivity of this method for different sources of samples. Real-time PCR method is highly sensitive and specific as reported in many studies all over the world, though, it is a time-consuming method and can survey only one target sequence in per run (Ponchel et al., 2003; Ramalingam et al., 2009).

But Compared to QPCR, MLPA is a low cost, fast, and technically uncomplicated method for the analysis of

Table 2. Demographic data of acute lymphoblastic leukemia patients

Variable		Frequency (Percent)
Sex	Female	18(40%)
	Male	27(60%)
Age	1-4	22(48%)
	5-10	17(37%)
	10-14	6(13%)
Mean of age	Female	6.0 ±2.7
	Male	5.8±3.4
NCI risk group	Standard risk	28(62%)
	High risk	17(37%)
Source of samples	BM samples	19(42%)
	PB samples	26(57%)
WBC count	>50,000	11
	<50,000	34

results. Moreover, in the future, MLPA could be applied to large CNAs screening. PCR and sequencing can be used for confirming mutation only in selected gene segments, but MLPA can be considered for screening a large area of genes, simultaneously.

CONCLUSION

Molecular screening is an essential test for comprehensive survey of disease, and provides complementary information for better diagnosis, treatment and follow-up of patients with acute leukemia. This approach may be useful as a criterion for measuring the effectiveness of new molecular tools such as genetic profiling. Considering differences between two methods, more clinical studies are required to find out the best one for our objectives. Our data clearly indicated that MLPA can be an attractive alternative method to other molecular and cytogenetic techniques that are now routinely used. Screening of critical genes in pathobiology of all cancers such as acute lymphoblastic leukemia could help early diagnosis in the early stages. It is especially useful when the inexpensive and available techniques are used everywhere. It has been established that early diagnosis has a positive impact on the prognosis.

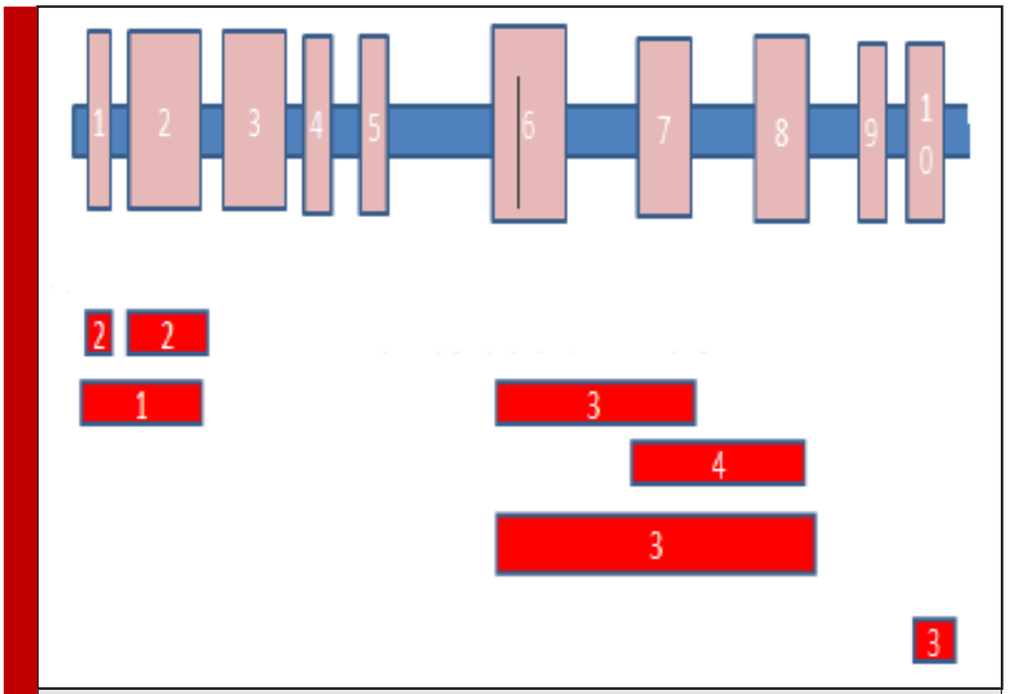

FIGURE 3. Schematic representation of the CNAs found in this study. PAX5 exons (1-10) are shown above. Numbers in the red shapes show the occurrence times for each rearrangement.

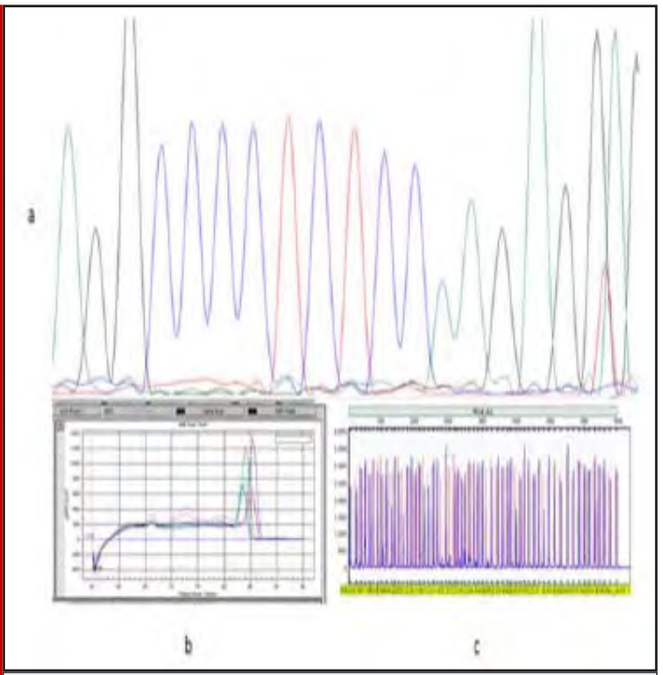

FIGURE 4. The comparison of the results of 3 methods; a: sequencing result, b: QPCR result, c: MLPA result.

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Effect of educational intervention based on health promotion model (HPM) on promoting behavior in safe delivery among Afghani pregnant women refugees in Sirjan, Iran

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ABSTRACT

Health promotion behaviors can promote physical and psychological health in individuals and population, especially in Afghan Pregnant Women. Therefore, this study performed to recognize the Effect of Educational intervention Based on Health Promotion Model (HPM) on promoting behavior in Safe Delivery among Afghan Pregnant Women in Sirjan city, 2016. This was a quasi- experimental study. 120 Pregnant Afghan Women attending health centers in Sirjan city were selected to participate in this study: (60 in intervention group and 60 in control group). The intervention was conducted over two sessions in 60 minutes. Data were collected by a validated and reliable questionnaire (39 questions) before intervention and three months after intervention. Data was analyzed by chi-square, Fisher's exact, paired t-test and independent t-test. The average scores of both groups indicated that health-promoting behavior, perceived self-efficacy, perceived barriers, perceived benefits, perceived social support and interpersonal norms, had no meaningful differences before the intervention. But after education, the average scores of all variables increased meaningfully in the intervention group ($P<0.001$). But, there was no increase or significant difference in the control group. HPM was effective in educating Afghan Pregnant Women. Therefore, it is suggested that HPM can be used to improve the safe delivery in Afghan women.

KEY WORDS: HEALTH PROMOTION MODEL, AFGHAN PREGNANT WOMEN, SAFE DELIVERY, SIRJAN

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INTRODUCTION

Maternal and infant mortality due to complications of pregnancy is a very important health indicator. Whatever the percentage of deliveries in non-sanitary and out-of-hospital increase, the mortality rate of infants and pregnant women and neonatal tetanus infection will increase too (Wax et al., 2010). Great deals of investment have been spent to reduce maternal and infant mortality rate and to reach the World Health Organization goal in the country, and also maternity and childbirth facilities have been built in various parts of Iran (Karyani et al., 2015).

One of the most vulnerable groups in this regard is refugees Afghan pregnant women. Decades of war and internal conflict in Afghanistan has led to migrate a large number of Afghans to neighboring countries, including Iran. One of the problems of these immigrants in Iran is the high rates of non-safe and non-sanitary delivery despite the availability of maternity facilities (Kalafi et al., 2002). Even in the Sirjan city in spite of the existence of maternity and women's specialists and well-equipped midwifery, 67% of Afghan women deliver babies at home in unsanitary conditions and by non-specialists, while this rate of giving birth at home is only 1% in developed countries (Sadeghi et al., 2015b). But in some developing countries, this ratio reaches to 50% or even more (Gloyd et al., 2001).

In the different studies, several reasons have been mentioned for giving birth at home, including high cost of delivery in the hospital, Fear of the hospital, husband's opposition, wrong culture, mother's emotional support at home and fear of cesarean section (Salehi and Pour, 2002).

The changing of this risky behavior in Afghan pregnant women requires proper education. The value of education depends on its Effectiveness and changing or creation of health behaviors, which is possible with the proper use of theories and educational models (Glanz et al., 2008). Theories identify the main factors that had influenced in question behaviors, specify the relationships between these factors, and outline the circumstances, the manner and the time of the occurrence of these relationships. Therefore, theories are useful in identifying the elements that should be considered as the main axis of interventions (Sadeghi et al., 2016). Health Promotion Pender Model is one of the comprehensive and predictive models which is used to study the health promoting behaviors and provides a theoretical framework for discovering affected factors in these behaviors (Pender, 2011).

The determinants of health promotion behaviors concepts in this model include: 1) individual experiences and Characteristics, 2) emotions and behavioral specific cognition, and 3) behavioral outcomes. The concept of individual experiences and Characteristics is a concept that directly or indirectly influences on the behaviors through cognition and behavioral emotions, including personal factors and previous related behaviors. While the concept of cognition and behavioral specific emotions directly influence on the behaviors and include constructs such as perceived benefits and barriers, perceived self-efficacy, behavioral emotions, interpersonal influences, and situational influences (Pender, 2011).

Professor Pender has identified models that have influenced on explanation of behavior in over 50% of the researches, including personal factors (perceived health status), perceived benefits, perceived barriers, perceived self-efficacy and interpersonal influences (Social support) (Pender, 2011).

Several studies have confirmed the effectiveness of this model. In this regard, a study by Morowati Sharifabad et al aimed at optimizing the health promotion model indicated that perceived self-efficacy was the most important determinant of health promotion behaviors and also perceived religious support was directly and indirectly have been effective by influencing on perceived self-efficacy, perceived benefits, perceived barriers, and perceived importance of health promotion behaviors (Morowati et al., 2005). The results of Maglione et al. showed that individuals with higher levels of social support, self-efficacy and with commitment of planning are more physically active (Maglione and Hayman, 2009). Considering the importance of the safe delivery and due to the high rate of delivery at Afghani homes; it seems that a similar study has not done in this regard. Therefore, the present study aimed to investigate the effect of educational intervention using the health promotion model in promoting behavior of Safe delivery among refugees Afghan Pregnant Women in Sirjan city in 2016.

MATERIALS AND METHODS

This quasi- experimental study was conducted in 2016. The research environment was the health centers of Sirjan city and the study population was Afghani pregnant women. Subjects were selected by simple random sampling. The sample size was calculated by using the ratio difference formula by considering the confidence level of 95%, a power of 80%, and about 3/3 differences in score before and after the intervention with a standard deviation of 7 and 52 subjects in per group, but the sample size was finally calculated as 60 subjects with consideration of 10 percentage of possible number of subjects dropping out in each group (Mohammadipour et al., 2015).

The subjects were selected based on the inclusion criteria which were as follows: Afghans, being pregnant in the third up to fifth month and resident of Sirjan city; the exclusion criteria were: without chronic disease such as pregnancy diabetes and high blood pressure, non-residence until the end of pregnancy and unwilling to participate in the study. Data collection tool was a self-administered questionnaire. The questionnaires were set anonymously and encoded and were arranged in three parts. The first part contains the demographic characteristics including 5 questions about age, education, occupation, number of pregnancies, and residence. The second part was related to the evaluation of the structures of the Pender Health Promotion Model. This part contains seven questions for perceived benefits, seven questions of perceived barriers, seven questions of perceived self-efficacy, six questions of perceived interpersonal norms, and six questions of perceived social support structures. The third part was related to the measurement of safe delivery behavior. The questions were scored as follows. questions about perceived benefits, perceived barriers, perceived self-efficacy, interpersonal norms and perceived social support were scored 1-3 by Likert's trilogy scale as agree (score 3) to disagree (score 1), The range of scores for perceived benefits, perceived barriers and perceived self-efficacy were from 7 to 21 and for interpersonal norms and perceived social support was from 6 to 18. Also, the assessments of safe delivery behavior were of yes/no type questions.

In this research the analyzing the validity of the content method was used for the analyzing the validity of the questionnaires. The questionnaire was approved by healthcare professionals to examine the issue's comprehensiveness. The re-test method was also used in the distance of 2 weeks to determine the reliability of the study. The correlation coefficient has calculated between the two-time responses as follows, for perceived benefits, 0.79, perceived barriers, 0. 77, perceived self-efficacy, 0.74, perceived interpersonal norms, 0.84 and perceived social support, 0.81.

The method was as follows the researchers randomly divided subjects into intervention and control groups after the selection them based on inclusion criteria. At first, the pre-test questionnaire was completed by the interviewer, then the education program was conducted for the intervention group. Educational content included the definition of pregnancy, maternity care, and also the benefits of safe delivery.The educational program was conducted for four groups of 15 subjects (60 subjects in intervention group), which consisted of two, one-hour training sessions in a one-month interval that were held in a lecture and group discussion. Also, an individual counseling was held for a pregnant woman and their family. The educational classes were held in health centers and health care homes. After three months of conducting the classes, post-test questionnaires were completed by the researcher for the patients in the intervention and control groups.

The current research has been approved by the Ethics Committee of the Kerman University of medical sciences (ethics code: IR.KMU. REC. 1396.11). All the participants were voluntarily and consciously participated in the study and they were given the assurance that the information has been collected only in order to be used for research and will remain confidential and the written testimonial had been gotten from all of the participants in order to participate in the study. Meanwhile, although the control group was not under educational intervention, but after completing the post-test, educations were provided to them with the same quantity and quality.

Data were analyzed by using Chi-square, Fisher's exact test, independent t-test and paired t-test through SPSS version 16. The significance level was considered less than 0.05.

RESULTS AND DISCUSSION

In this study, 120 participants were randomly divided into two groups, intervention (n = 60) and control (n = 60) then the mean and standard deviation of the ages of subjects in the study and control groups were analyzed and they were respectively 24.83 ± 9.43 and 25.12 ± 9.26 years old. This difference based on independent t-test did not show significant differences in both intervention and control groups (P = 0.426). Also other demographic characteristics of the population did not show any significant differences between the two groups (Table 1).

The results showed that there wasn't any significant difference between the mean scores of perceived benefits, perceived barriers, perceived self-efficacy, interpersonal norms and perceived social support before the intervention in the intervention and control groups, but after the intervention, the differences were significantly clear and Scores were increased (Table 2).

The statistical results showed that in the study group, there was a significant increase between the mean scores of perceived benefits, perceived barriers, perceived self-efficacy, interpersonal norms and perceived social support before and after the intervention, and also the educational intervention was effective (Table 3) .

The results also showed that the safe delivery behavior in the intervention group have increased from 23% to 57% after the educational intervention.

Afghan women carry out childbirth at home and in unhealthy conditions, due to traditional beliefs and misconceptions of them and their followers, which unfortunately can lead to the death of mother or infant and may lead to complications such as neonatal tetanus.

Table 1. Comparing some variables among the refugees Afghan Pregnant Women in Sirjan city in the control and intervention groups

Variable		intervention Group (n= 60) Number (%)	Control Group (n= 60) Number (%)	*p-value
Occupation	Housewife	55 (50.4)	54 (49.6)	0.251
	Employed	5 (45.4)	6 (54.6)	
Pregnancy status	First pregnancy	12 (52.1)	11 (47.9)	0.925
	More than one pregnancy	48 (49.4)	49 (50.6)	
Education	Literate	10 (47.6)	11 (52.4)	0.712
	illiterate	50 (50.5)	49 (49.5)	
Residence	City	28 (49.1)	32 (50.8)	0.283
	Village	29 (50.9)	31 (49.2)	

*Chi-square test

Table 2. The mean scores of refugees Afghan Pregnant Women in Sirjan between the control and intervention groups regarding Safe Delivery before and after the intervention

	Before intervention (n=60)		p-value*	After intervention (n=60)		p-value*
	Intervention group Mean± SD	Control group Mean± SD		Intervention group Mean± SD	Control group Mean± SD	
perceived benefits	12.14±2.21	11.94±1.87	0.212	18.42±0.64	12.02±1.56	<0.001
Perceived barriers	18.82±2.72	18.41±3.09	0.367	14.77±1.46	18.25±3.11	<0.001
perceived self-efficacy	12.94±3.23	12.64±4.37	0.055	17.18±1.39	12.29±3.85	<0.001
interpersonal norms	10.19±2.61	10.71±2.83	0.412	14.21±1.67	11.14±2.78	<0.001
perceived social support	7.24±2.43	7.58±2.67	0.216	14.12±3.73	7.68±2.83	<0.001

*Independent t-test

Table 3. The comparison of the variables. The mean scores of refugees Afghan Pregnant Women in Sirjan between the control and intervention groups regarding Safe Delivery before and after the intervention

	Intervention group (n=60)		p-value*	Control group (n=60)		p-value*
	Before Intervention Mean± SD	After intervention Mean± SD		Before Intervention Mean± SD	After intervention Mean± SD	
perceived benefits	12.14±2.21	18.42±0.64	<0.001	11.94±1.87	12.02±1.56	0.021
Perceived barriers	18.82±2.72	14.77±1.46	<0.001	18.41±3.09	18.25±3.11	0.424
perceived self-efficacy	12.94±3.23	17.18±1.39	<0.001	12.64±4.37	12.29±3.85	0.356
interpersonal norms	10.19±2.61	14.21±1.67	<0.001	10.71±2.83	11.14±2.78	0.172
perceived social support	7.24±2.43	14.12±3.73	<0.001	7.58±2.67	7.68±2.83	0.481

*Paired t-test

Therefore, in order to control this important and fundamental dilemma, we evaluated the relevant training based on the health promotion model to promote safe delivery behavior.The findings showed significant increase in the safe and hygienic delivery of Afghan women from 23% to 57% in Sirjan city.

In the health promotion model, in the intervention group, all of the constructs of the model showed significant differences after the intervention.

In the health promotion model, perceived benefits are as a direct stimulus action of behavior and the adoption of an action to prevent the problem or action on a health behavior are dependent on its perceived benefits (Pender, 2011). The most important perceived benefits of safe delivery of Afghan pregnant women respectively included the baby's health, their health, and the prevention of unwanted childhood complications at home. In the present study, the average of perceived benefits scores of patients in the intervention group has increased significantly than in the control group after the education, which was consistent with the results of similar studies (Morovati, 2007, Leslie et al., 2000, Sadeghi et al., 2014). But the results of the study of Ghaffari et al was inconsistent to the present study and did not indicate an increase in the average score of perceived benefits (Ghafari, 2007).

In the present study, financial problems, lack of medical insurance and lack of access to facilities were respectively the most important obstacles in the target group. The significant reduction in the average score of perceived barriers after educational intervention and inter-sectional collaboration in the health care system indicates that the education has a positive effect on removing the perceived barriers in the intervention group. The studies of Nowroozi et al. (Noroozi et al., 2011) and Karimi et al. (Karimi and Eshrati, 2012), which were conducted with this model, indicate that educational intervention are effective in reducing perceived barriers.

Self-efficacy is referred to a person's confidence to his ability in performing an action (Bandura, 2006). In the present study, the average of self-efficacy score of the experimental group after the intervention was significantly increased, which were consistent with the studies of Morowati et al. (Morowati et al., 2005) and Sadeghi et al. (Sadeghi et al., 2015a), but they contradicted by the findings of Kinsler et al. (Kinsler et al., 2004).

Also, in the study, after the education, the average scores of the interpersonal norms of the intervention group were significantly higher than in the control group, which was consistent with the results of the studies of Chenari et al (Chenary et al., 2013) and Nosratabadi et al (Nosratabadi et al., 2015). In the present study, perceived social support was considered as the most important determinant of the behavior of delivery at home, which this issue was arisen from the influences of family and relatives on this unhealthy behavior. But after educational sessions for the participants and their relatives, the average score in the intervention group was significantly higher than in the control group, which is consistent with the results of conducted studies with this model (Lusk et al., 1997, Norouzi et al., 2010). According to this research and the findings, it is suggested that a precise educational program should be carried out based on the health promotion model for Afghan pregnant women, their spouses and their relatives, and planners should train them by using individual and group educational methods, in order to solve their financial and health problems, and to prevent the risks of unsafe deliveries at home.

One of the limitations of this study was the problem of Afghan women's language and their accent, which was solved by Afghan inquirer and educator, as well as Unwillingness of some of the women to participate in the study, which it was solved by convincing them through local clerks and Afghan elders. Another limitation of the study was the financial and insurance problems of Afghan women, which it was reduced by inter-agency co-ordinations.

CONCLUSION

The results of this study indicates the impact of educational intervention based on the health promotion model on increasing the safe delivery of pregnancy in Afghan pregnant women in Sirjan city. education and intervention based on the health promotion model is helpful and effective to enhance awareness, to change the attitude and to adopt preventive behaviors for unsafe delivery at home.

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AUTHORS' CONTRIBUTIONS

RS helped design the study, carried out data collection, data analysis, and drafted the manuscript. VM carried out the statistical analysis and interpretation. MS conceived the study, supervised the data collection and analyses, and helped draft the manuscript. BB edited and commented on the final draft. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Effect of soil tillage systems on chickpea yield and moisture of soil

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ABSTRACT

Sustainable agriculture is now considered as an important factor in the development of modern agriculture. Sustainable agriculture emphasizes the long-term yield stability with the least adverse impact on the environment. So tillage is a very important factor in agricultural economics. The aim of this study was to investigate the effect of tillage on chickpea yield in split strip plots based on a randomized complete block design with three replications in two growing years of 2014-2015 and 2015-2016 in Mahidasht located in Kermanshah. In this research, it was found that in the first year, the results of analysis of variance showed that the effect of tillage was significant only on grain yield at 5% level. However, the effect of tillage on moisture content was not significant in the studied properties. In the second year of experiment, the tillage factor also had a significant effect on grain yield, biological yield, and moisture content at the depth of 0-20 cm at 5% level, and it was significant on moisture content at the depth of 20-40 cm at second phase at 1% level. Since the yield of the crop was lower in no tillage than in other cases, but the use of no tillage has caused that there is no place for agricultural equipment and machinery in the farm which caused density in soil. It should be noted that these machines and equipment are so expensive,this issue is very important in economic terms; therefore, the use of no tillage system is proposed.

KEY WORDS: NO TILLAGE, LOW TILLAGE, CONVENTIONAL TILLAGE, MOISTURE, CHICKPEA

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INTRODUCTION

In today's world, agriculture has faced the enormous challenges; soil erosion significantly reduces the yield of crops.Shortage of water and an increase in the price of fuel and fertilizer will increase production costs. Increasing demand for food as a result of the unprecedented growth of the population in recent decades has created a major challenge for researchers in the agricultural sector. The limitation of susceptible lands has caused that most of the strategies have not been led to increase yields per unit area Beans are one of the nutrient food for human and animal. In the agricultural systems of the world in rotation with other crops and nitrogen fixation, atmosphere in co-existence with bacteria provides the major part of the nitrogen needed for the next crops, (Sadeqipour, 2005, Goldani and Rezvani Moqaddam, 2007 and Afzali and Javaheri, 2013).

Soil tillage affects the important properties of soil such as temperature, moisture, and soil density. Thus, the correct use of tillage system can be the suitable strategy for plant growth and the optimal yield. The conventional tillage prepare artificial bed for the plant growth through the breakdown of soil impermeable layers, cleansing of soil surface from plant debris and discontinuing the life cycle of weeds, insects and diseases. But these systems not only require a lot of energy, but also, in the long run, these may destroy the physical properties of the soil and erode it. While water permeability in soil increases in low tillage systems due to increased organic matter and earthworm activity compared with conventional tillage system. Also, the use of low tillage systems and soil freeze will reduce the cost of energy consumption, reduce erosion and soil degradation, (Habibzadeh, 2006, Rasouli and Abbaspour, 2008 and Heidari, 2011).

Tillage operations are an inseparable part of the crop production cycle. The purpose of the tillage operation is the primary and main work on the soil, which is planned to reduce soil resistance, cover vegetation cover, and homogenizing its structure (Shafiei, 1992; Shafiei, 1995). According to Papendick and Parr (1997), due to organic matter depletion in dryland areas, the agricultural system will not be sustainable based on conventional tillage. Chen et al. (2006) concluded that the lack of moisture in the surface layers of the soil may cause the plant derives its moisture from the deeper layers of the soil that the essential nutrients are low. Thus, the plant suffers from nutrient stress; the sum of these factors reduces the plant size and existing photosynthetic reserves to fill the pods, and ultimately, it reduces the plant's yield.

Chassot et al. (2011) found that the surface of soil in no tillage is usually colder and wetter, and bulk densityis higher than conventional tillage. This has had an effect on the growth of chickpea root and the absorption of nutrients. Amini and Movahedi Naeini (2013) concluded that reducing the yield of products in no tillage system is directly affected by more mechanical soil resistance or lack of moisture and access to nutrients. By increasing the special surface of soils and thinning of clay soils, their cement property is strengthened and the mechanical strength of the soil increases. Even in wet conditions, the soil of the test site with a high surface has a high mechanical strength because, due to the fineness of the clay, the velocity of inflation and its equilibrium are very slowly absorbed by the waterwhich leads to a clogging between the soil components and mechanical strength. Long-term studies in the semi-arid region of the United States regarding field management by using conventional tillage, no tillage, and low tillage methods indicated that the weed control in no tillage system using herbicides only increased the wheat grain yield and the soil moisture. The yield of tillage is in the middle of the conventional and no tillage systems (Norwood and Currie, 1997).

MATERIAL AND METHODS

This research was carried out in dryland conditions in two separate experiments for chickpea in split plots (split plow split plots) based on a randomized complete block design with three replications in two growing years of 2014-2015 and 2015-2016 in Mahidshat which is located in Kermanshah. Three levels of tillage include: 1- conventional tillage (moldboard plowing), 2- low tillage (chisel plowing) and 3- no tillage. The experiment is carried out in a field that was cultivated the previous year. The area of each experimental plot (5 x 10 m) is 50 square meters and each replicate includes 18 sub plots and the distance is 10 meters to allow the tractor to pass. The total number of plots in this experiment will be 108. The amount of soil organic matter is measured before and after the project at a depth of 0-20 cm. To determine the percentage of soil moisture content at flowering time and harvesting, samples were taken from the depth of 0-20 cm and 20-40 cm. In the beginning of April, weeds are removed manually.

RESULTS AND DISCUSSION

Analysis of variance showed that the effect of tillage was significant on yield only at 5% level. However, the effect of tillage on moisture content was not significant in any of the traits (Table 4-1).

SECOND YEAR EXPERIMENT OF CHICKPEA

In the second year of experiment, the effect of tillage was significant on grain yield, biological yield, moisture

Table 4.1. Analysis of variance of the first year to investigate the effects of tillage onchickpea

		Mean of squares				
Sources of changes	df	Weight of 100 seeds	Number of pods per square meter	Number of seeds per pods	Grain yield	Biological yield
Replication	2	1.00	22.67	7.25	21072	224437
Error 1	4	2.02	5.77	1.64	11207	35285
Tillage	2	1.66	46.07	0.89	51799*	210352
Error 2	4	0.39	9.55	1.09	5820	232341

*and
**indicate the significance level at 1 and 5, respectively.

Table 4.2. Analysis of variance of the first year to investigate the effects of moisture on chickpea

		Mean of squares			
Sources of changes	df	Moisture content at the depth of 0-20 cmphase 1	Moisture contentat the depth of 20-40 cmphase 1	Moisture content at the depth of 0-20 cm phase 2	Moisture content at the depth of 20-40 cmphase 2
Replication	2	3.21	19.17	8.42	55.68
Error 1	4	0.50	2.72	2.19	16.99
Tillage	2	2.97	0.41	3.28	0.96
Error 2	4	1.36	0.91	1.51	1.66

*and
**indicate the significance level at 1 and 5, respectively.

content at the depth of 0-20 cm at 5% level, it was also significant on moisture content at the depth of 20-40 cm at the second phase at 1% level.

COMPARISON OF THE MEAN OF THE MAIN LEVELS OF THE FACTORS STUDIED IN CHICKPEA TWO-YEAR COMPOSITE ANALYSIS

The result of the comparison of the average levels of tillage is shown in Table 4-5 and 4-6. As it can be seen, the tillage factor had only significant effect on 100-seed weight and moisture content at the depth of 20-40 cm on the second phase; and it had no significant effect on other properties (Table 4-4, 4-5).

The tillage factor was only significant on the characteristics of 100 seed weight and leaf area index and was not significant on other characteristics. Mudak et al. (2001) in a seven-year trial on wheat yield reported that there is no significant difference between conventional tillage systems and no tillage. Simon et al., (2009) reported that soil tillage has an impact on most soil characteristics such as temperature, moisture distribution, and soil density, and the proper selection and implementation of an appropriate tillage system can provide a suitable bedding for the seed, and ultimately lead to optimal yield (Hemmat & Eskandari, 2006).

Although conventional tillage systems provide a good basis for plant growth by breaking the impermeable lay-

Table 4.3. Analysis of variance of the second year to investigate the effects of tillage on chickpea

		Mean of squares				
Sources of changes	df	Weight of 100 seeds	Number of pods per square meter	Number of seeds per pods	Grain yield	Biological yield
Replication	2	0.97	238	12	21358	261282
Error 1	4	0.75	112	33	43026	69751
Tillage	2	4.36	18	10	155361	724726*
Error 2	4	1.05	15	34	1384	91214

*and
**indicate the significance level at 1 and 5, respectively.

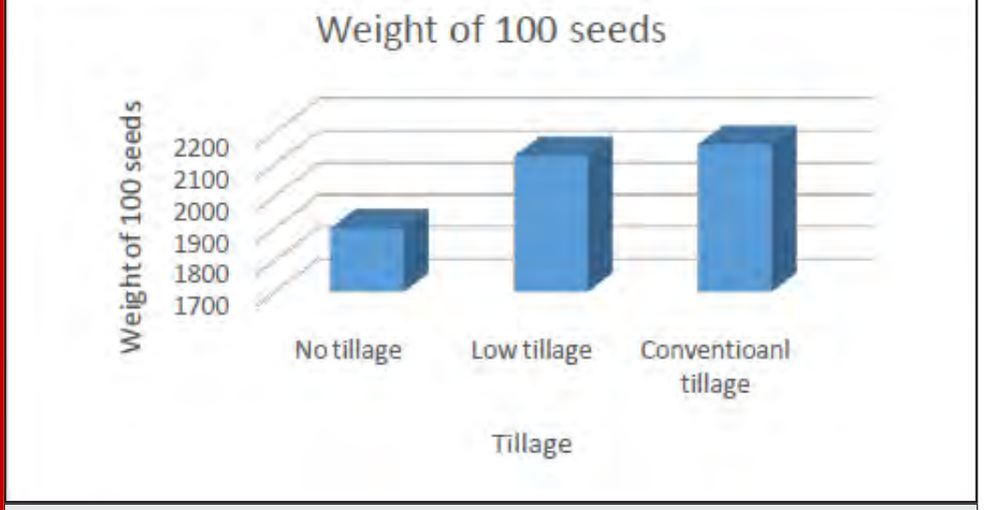

FIGURE 1. Effect of tillage on grain weight

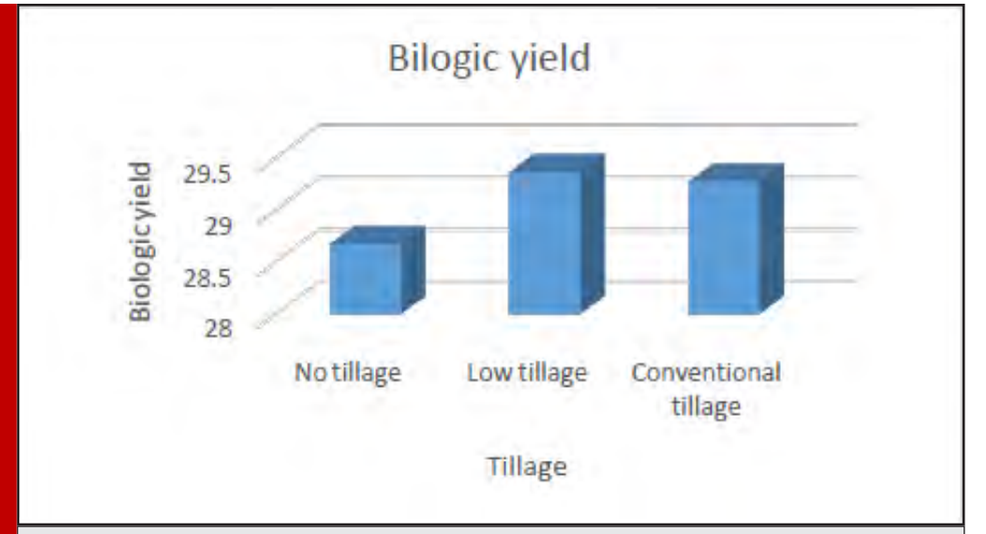

FIGURE 2. Effect of tillage on biological yield

Table 4.4. Analysis of variance of the second year to investigate the effects of moisture on chickpea

		Mean of squares			
Sources of changes	df	Moisture content at the depth of 0-20 cm phase 1	Moisture content at the depth of 20-40 cm phase 1	Moisture content at the depth of 0-20 cmphase 2	Moisture content at the depth of 20-40 cmphase 2
Replication	2	5.09	7.95	0.17	0.7
Error 1	4	0.81	5.75	0.38	0.57
Tillage	2	6.35	4.30	*1.32	**5.13
Error 2	4	2.56	2.09	0.16	0.20

*and
**indicate the significance level at 1 and 5, respectively.

Table 4.5. Comparison of the mean of tillage effect

Characteristics						
Factor		Weight of 100 seeds	Number of pods per square meter	Number of seeds per pods	Grain yield	Biologic yield
Year	First year	10.16[b]	19.63[a]	27.23[b]	469.76	1248[b]
	Second year	30.93[a]	25.41[a]	30.97[a]	829.06	2876[a]
Tillage	0	20.70[a]	21.53[a]	28.67[b]	0	1900[b]
	1	20.73[a]	22.06[a]	29.36[a]	1	2125[ab]
	2	20.20[a]	23.96[a]	29.27[a]	2	2161[a]

In each column and for each factor, the meanings that have at least one letter are not significantly different at the 5% probability level.
*: Tillage 0, 1 and 2 respectively means non-tillage, low tillage, and conventional tillage.

Table 4.6. Comparison of the mean of moisture effect

Characteristics					
Factor		Moisture content at the depth of 0-20 cm phase1	Moisture content at the depth of 20-40cm phase1	Moisture contentat the depth of 0-20 cm phase 2	Moisture content at the depth of 20-40cm phase 2
Year	First year	13.90[b]	26.96[b]	11.72[a]	15.34[a]
	Second year	15.51[a]	30.53[a]	9.23[b]	12.20[b]
Tillage	0	1508[a]	28.43[a]	b10.25	13.47[a]
	1	14.89[a]	a28.55	10.54[a]	14.07[a]
	2	14.14[a]	a29.25	a10.64	13.76[a]

In each column and for each factor, the meanings that have at least one letter are not significantly different at the 5% probability level.
*: Tillage 0, 1 and 2 respectively means non-tillage, low tillage, and conventional tillage.

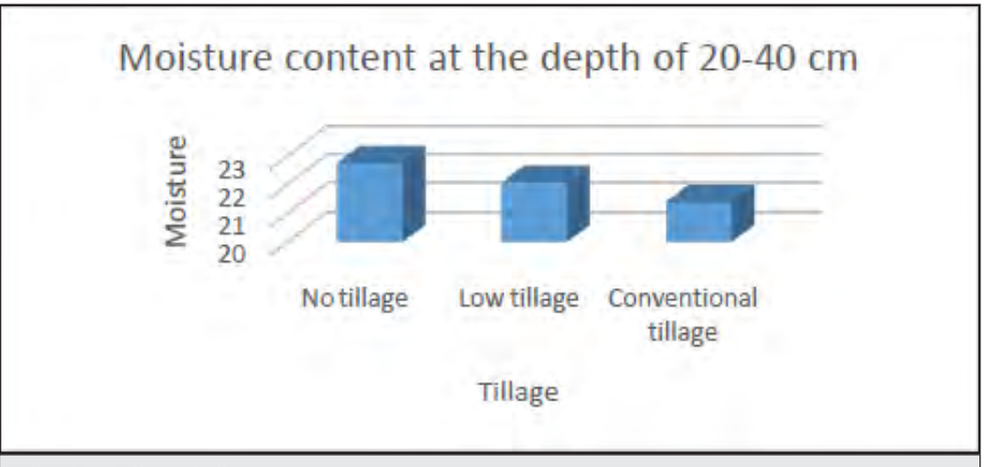

FIGURE 3. Effect of tillage on moisture

ers of soil and cutting the weed life cycle, pests and diseases (Mulumba & Lal, 2008), but these systems also require a lot of energy and In the long term, they destroy the physical properties of the soil (Sharma et al., 2011). Also, the use of low tillage and non-tillage systems reduces the costs of energy consumption, it decreases erosion and soil degradation (Katsviro 2002; Barzegar et al. 2004).

Astillage machines are heavy and large require 120 horsepower tractors to pull; these tractors cause density in the soil when traveling on the farm. So it's economical to use organic farming systems for ordinary farmers, and any farmer can use no tillage system.Since the yield in no-tillage compared to other system is lower, but there is no use of agricultural equipment and machines in no tillage system, this is so important in terms of economic.

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Dental
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The role of different impression methods for complete denture prosthodontics

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ABSTRACT

The aim of the study was to determine dentist's desire for role of different impression methods for complete denture prosthodontics.The 1378 questioners form randomly distributed among dentists. The form included questions related about their idea for application and effect of boarder modeling materials in complete denture prosthodontics. Among the total forms, 657 forms fully filled, signature and returned by the dentists. **Results:** Acceding to the data, the highest percent of the dentists included in this study was graduated 11-15 years ago (28%). Also, 73% of the dentists always apply two complete dentures prosthodontics. 73% of the dentists answered for always use administration of special trays. 64% of the dentists answered that they always use impression before border modeling and 25 % of them never use this. 89% of the dentists use compound material for border modeling. 64% of the patients use zinc oxide and eugenol (ZOE) and the 25% use Alginate. Also, poly ether and poly sulfate were desired by 10.5 and 0.5% of the dentists, respectively. As seen there are different ideas among dentist for different impression methods for complete denture prosthodontics.

KEY WORDS: IMPRESSION METHODS, MATERIAL, COMPLETE DENTURE PROSTHODONTICS

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INTRODUCTION

An impression is a record, a facsimile of mouth tissues taken at an unstrained rest position or in various positions of displacement (Devan, 2005). In the case of an edentulous arch, this requires a unique combination of managing movable soft tissue commensurate with integrating different materials and a technique for accurate reproduction (Petrie et al., 2005). Stability of complete lower dentures has challenged dentists and patients alike. In particular, "flat lower ridge" is associated with difficulties in providing successful dentures (McCord et al. 1992). Resorption rates vary from patient to patient and some authors have postulated several etiological factors related to residual (alveolar) ridge resorption, ranging from localized pressure to systemic factors (Likeman, 1997). Making accurate final impression for complete dentures is a multistage process that involves a preliminary impression, a customized final impression tray and a final border impression (Zarb et al. 1990).

It is important to thoroughly examine the patient's mouth and select the most appropriate impression technique (Suenaga et al. 1997). A major requirement for final impression of complete dentures is to develop the peripheral contours to accommodate normal muscular function and to ensure peripheral adaptation without allowing air penetration between the future denture base and the mucous membrane (Daou, 2010). Differences are noticeable between the materials and methods currently used by dentists for final impressions in complete denture prosthodontics (Daou, 2010).

Even though the current generation of impression materials provides alternatives, making an initial impression still can be difficult when patients have significant resorption (Pyle, 1999). The concept of molding the periphery of complete denture prosthesis to the surrounding musculature has been accepted and taught for about past decades (Drago, 2003). Anecdotal evidence suggests that the impression techniques used in general dental practice may vary from those taught at dental schools (Drago, 2003). So, the aim of the study was to determine dentist's desire for role of different impression methods for complete denture prosthodontics.

MATERIAL AND METHODS

This cross sectional study was done using questioners on dentists during 2014-15. The 1378 questioners form randomly distributed among dentists. The form included questions related about their idea for application and effect of boarder modeling materials in complete denture prosthodontics. Among the total forms, 657 forms fully filled, signature and returned by the dentists. Then the data processed using excel software using Microsoft office ver. 2010 and presented as frequency and percent.

RESULTS AND DISCUSSION

The result of the different impression methods for complete denture prosthodontics is presented in figs. 1-6. The frequency of the dentists included into the study based on their years of graduate is presented in figure 1. As seen the highest percent of the dentists included in this study was graduated 11-15 years ago (28%) and only 11% was graduated less than 5 years

The frequency of the dentists answer for application of 2 complete dentures prosthodontics is presented in figure 2. As observed 73% of the dentists always apply two complete dentures prosthodontics, initial and final while only 3 % sometimes used this.

As seen in figure 3, 73% of the dentists answered for always use administration of special trays and only 8 % of the dentists never used special trays.

Also, 64% of the dentists answered that they always use impression before border modeling and 25 % of them never use this (figure 4).

Additionally, 89% of the dentists use compound material for border modeling while 11% of the dentists desire to use poly ether materials (figure 5).

Furthermore, 64% of the patients use ZOF and the 25% use Alginate. Also, poly ether and poly sulfate were desired by 10.5 and 0.5% of the dentists, respectively (figure 6).

As seen there are different ideas among dentist for different impression methods for complete denture prosthodontics. Also, there was different for materials used for complete denture prosthodontics among dentists. Since the last decade, several investigators have recommended using newer elastomeric materials such as polyvinylsiloxane and polyether for final impressions to replace the older and more traditional materials (Petrie et al., 2005). Four basic types of elastomer impression materials are currently in use in the dental profession such as silicone rubbers which polymerize by a condensation reaction, polysulfide (mercaptan) rubbers, polyethers and silicones which polymerize an addition reaction. The latter have been introduced relatively recently and are also called polyvinylsiloxanes (Lacy et al., 1981).

There are many materials for the final impression, such as gypsum, zinc oxide eugenol (ZOE) paste, polysulfide rubber, polyether, polyvinyl siloxane and alginate. Preferences vary much among dentists. However, there is no evidence that one technique or material produces better long term results than another (Duncan and Taylor, 2001). Many general practitioners use a single alginate impression as the definitive impression for

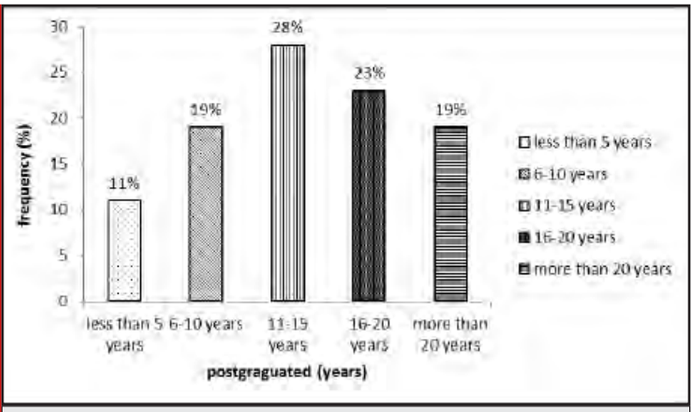

FIGURE 1. The frequency of the dentists included into the study based on their years of graduate

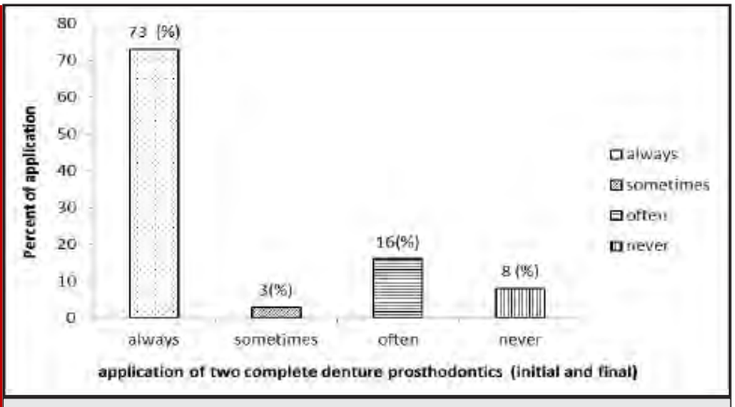

FIGURE 2. The frequency of the dentists answer for application of two complete dentures prosthodontics (initial and final)

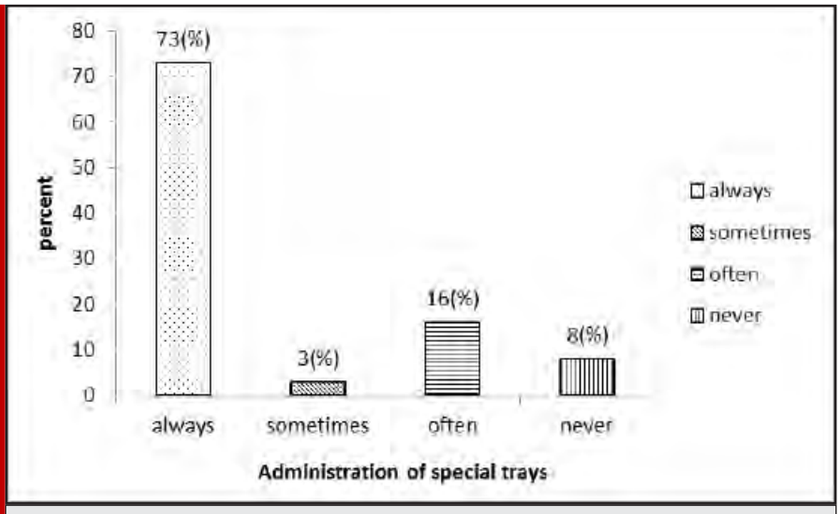

FIGURE 3. The frequency of the dentists answer for administration of special trays

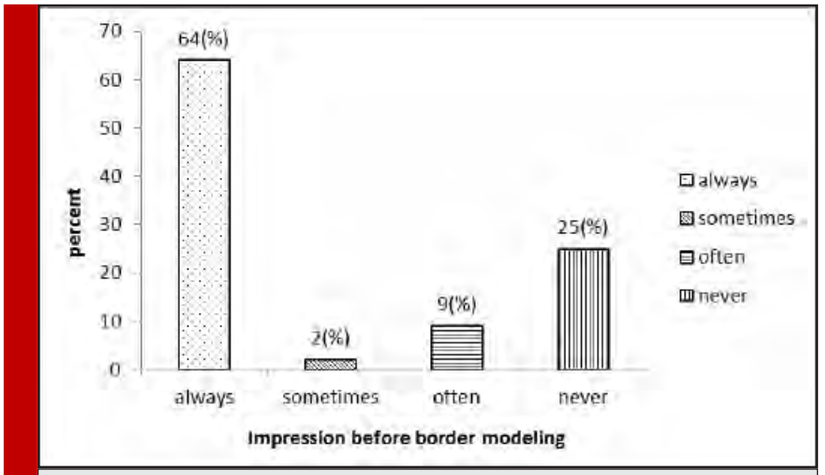

FIGURE 4. The frequency of the dentists answer for Impression before border modeling

the construction of complete dentures, which conflicts with the teaching in practically all dental schools. It is, therefore, of interest that a randomized controlled trial found neither patient-assessed nor dentist-evaluated differences between dentures fabricated according to a traditional or a simplified method. The simple technique used alginate in a standard tray for the definitive impression, whereas the traditional technique included an individual tray with border molding and polyether for the final impression (Kawai et al., 2005). Although impression materials differ in many aspects and a variety of techniques exist in taking the impressions, there is no evidence to conclude that the clinical long-term outcome of dentures fabricated using varying materials and methods would differ significantly. These and other aspects of variation in methods and techniques are discussed in a review of an evidence base for complete dentures (Carlsson, 2006).

It is reported complete dentures fabricated with the conventional method that included a prelimi- nary impression made using alginate in a stock tray and subsequently a final impression made using silicone in a border moulded custom tray resulted in higher general patient satisfaction (Lepe et al. 2002). Currently, there are a number of issues with prosthodontic protocols that may provide underlying reasons why clinically significant differences are not produced in randomized controlled trial. Prosthodontic trials can have numerous

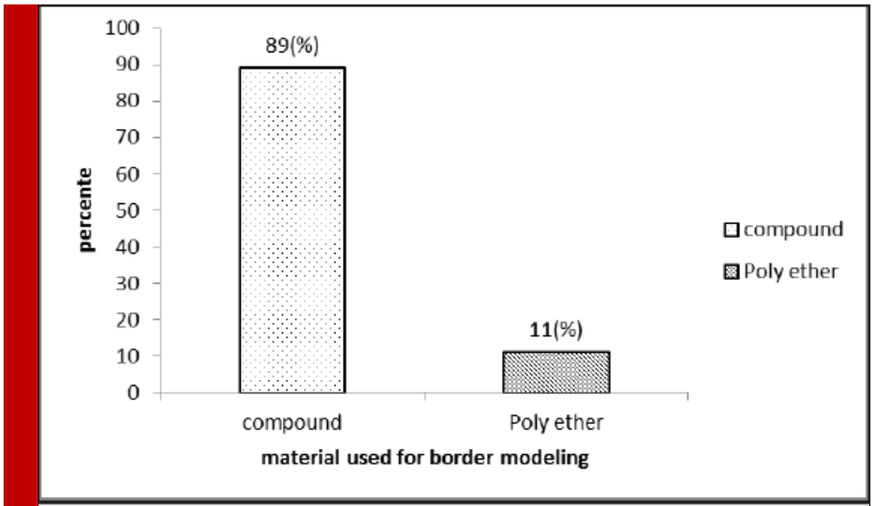

FIGURE 5. The frequency of the dentists answer for material used for border modeling

specific confounding variables such as patient related factors (ridge form, saliva flow, mucosal quality, patient expectation, psychological profile, perceived aesthetics), technical construction factors (occlusal form, impression technique, processing methods, different technicians/ technical procedures, the full use, or not, of the recorded sulcus depth) and dentist related factors (ability, education, number of clinicians, velocity of seating of the impression) (Dillon et al, 2008).

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Biotechnological Communication

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BBRC Bioscience Biotechnology Research Communications

Influence of different spacing and cultivars on yield components and biochemical parameters of onion (*Allium cepa L.*)

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ABSTRACT

The investigation was undertaken to determine the effect of spacing and cultivars on economic horticultural traits of onion. In trail different spacing was taken 7.5 × 10 cm, 10 × 10 cm, 12.5 × 10 cm and 15 × 10 cm. Three varieties viz. Agrifound Light Red, NHRDF Red-3 and NHRDF L-28 were used for study. The layout of experimental field was laid down in Factorial Randomized Block Design with 3 replications. It is clearly revealed that the significantly contrary, yield ha^{-1} was the highest (404.14 q ha^{-1}) at closer spacing (10 × 10 cm) and the lowest was (362.47 q ha^{-1}) at wider spacing 15 × 10 cm. The weight of individual bulb of onion (49.54 g) was increased with the wider spacing (12.5 × 10 cm). The bulb length (6.63 cm), diameter (6.97 cm) and number of scale per bulb (8.00) also the same trend in widest spacing (15 × 10 cm). The interaction of spacing and different cultivars had influenced significantly on total soluble solids and ascorbic acid of onion bulb. Significant effect was found among the varieties for total soluble solids, ascorbic acid, reducing sugar. Studied highest total soluble solids (13.960Brix), ascorbic acid (10.03), reducing sugar (8.50), non reducing (9.70%) and total sugars (17.70%) respectively.

KEY WORDS: ONION, SPACING, CULTIVARS, GROWTH AND YIELD

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INTRODUCTION

Onion is a bulbous herbaceous biennial vegetable crop which belongs to family *Alliaceae* and widely grown as with cross-pollinated and monocotyledonous behavior having diploid chromosomes 2n=16 (Bassett, 1986). Onion is the most important bulb crop cultivated commercially in most parts of the world. The crop is grown for consumption both in the green states as well as in mature bulbs. It is valued for its bulbs having characteristics odour, flavor, and pungency, which is due to the presence of a volatile oil-allyl-propyl-disulphide, (Kantona *et al.,* 2003 and Habtamu *et al.,* 2016 and Kishor *et al.,* 2017).

Onion is the richest source of flavonoids in the human diet and flavonoid consumption has been associated with a reduced risk of cancer, heart disease and diabetes. In addition it is known for anti bacterial, antiviral, anti-allergenic and anti inflammatory potential. Even though the crop has great contribution both in economic and health issues, its production and productivity is not scaled to the required level. This is because use of appropriate agronomic management practices and improved technology inputs are still not highly used which have undoubted contribution in increasing crop yield potential. One of the important measures to be taken in increasing the productivity of onion is determining spacing for each agro-ecology since full package of information is required for each growing region the country to optimize onion productivity (Gupta, et al., 1994). Proper spacing ensures optimum plant growth through adequate utilization of moisture, light, spacing and nutrients (Zubeldia and Gases, 1977). The control of plant spacing is one of the cultural practices to control bulb size, shape and yield (Geremew et al., 2010). The higher yield and better control of over or under bulb size bulb size could be obtained if plants are grown at optimum density. Total bulb yield can be increased as population density increase (Kantona et al., 2003 and Habtamu et al., 2016 and Kishor et al., 2017).

Several researchers in many countries have shown that varieties and plant spacing had profound effects on the growth and yield of onion (Pandey *et al.,* 1991; Bhonden *et al.,* 1995 and Kumar *et al.,* 1998, Kishor *et al.,* 2017). Considering the above stated situations, the present study was undertaken to determine the effect of spacing on growth and yield of different cultivars of onion under Lucknow conditions (*Allium cepa l.*).

MATERIAL AND METHODS

The experiment was conducted at Horticulture Research Farm-II of Babasaheb Bhimrao Ambedkar University, Vidya Vihar Rae Bareli Road Lucknow (UP) during November 2015 to April 2016 under subtropical condition. The experiment site lies about 260 56 N latitude and 800 52 E longitude at an altitude of 111 m above sea level. The area experiences rainfall that stretches from April to October with the main rainy season from June to early September. The area receives average rainfall between 800-1000 mm with annual and maximum temperature ranging from 15 to 300C. Three varieties viz. Agrifound Light Red, NHRDF Red-3 and NHRDF L-28 and four plant spacing such as 7.5 × 10 cm, 10 × 10 cm, 12.5 × 10 cm and 15 × 10 cm were used for study. The experiment was laid out in Factorial Randomized Block Design (RBD) and replicated in thrice. Standard analytical methods were followed for recording various parameters. The observation was made on the following parameters yield kg/plot, yield kg/ha, weight of bulb, bulb length (cm), bulb diameter (cm), number of scale per bulb, total soluble solids (0Brix) was measured with the help of an Erma hand refractometer and were corrected using standard reference table and express in terms of (0Brix) at 200, ascorbic acid (mg/100g) Ascorbic acid content was determined by diluting the known volume of juice with 3% meta-phosphoric acid and titrating with 2,6- dichlorophenol-indo-phenol solution, reducing sugar (%), non reducing sugar (%) and total sugars (%) were determined by titrating the sample against Fehlings solution using methylene blue as an indicator. All the parameters were collected from five randomly selected plants of each treatment. On set of the Rabi season these healthy bulb uniform shape and size were selected and transplanted well prepared field Statistical analysis of the data obtained in different set of experiments was calculated following the standard procedure as stated by (Panse and Sukhatme, 1989).

RESULT AND DISCUSSION

YIELD COMPONENTS

The results obtained during the investigation in respect to yield components parameters viz., yield kg/plot, yield kg/ha, weight of bulb, bulb length (cm), bulb diameter (cm), number of scale per bulb, total soluble solids (^{0}Brix), ascorbic acid (mg/100g), reducing sugar (%), non reducing sugar (%) and total sugars (%) Table 1. The interaction effect of spacing and different cultivars had influenced significantly on the parameters. The maximum yield (7.34 kg/plot and 489.77 q ha^{-1}) was recorded from the variety Agrifound Light Red with spacing 10 × 10 cm followed by the variety NHRDF Red-3 (6.88 kg/plot and 458.77 q ha^{-1}) with spacing 7.5 × 10 cm. The minimum yield (4.67 kg/plot and 311.44 q ha^{-1}) was recorded from the variety Agrifound Light Red with spacing 12.5 × 10 cm. While the heaviest bulb (57.60 g) was recorded from

the variety NHRDF L-28 with spacing 12.5 × 10 cm followed by variety NHRDF L-28 with spacing 15 × 10 cm (55.00 g) and lightest bulb was obtained from the variety Agrifound Light Red with 7.5 × 10 cm. While the biggest bulb diameter (7.06 cm) was recorded from the variety NHRDF Red-3 with 15 × 10 cm followed by the variety Agrifound Light Red with spacing 15 × 10 cm (6.86 cm).

The lowest bulb diameter (5.01 cm) was recorded from the variety Agrifound Light Red with 7.5 × 10 cm. While the length of bulb and number of scale per bulb was not significantly influenced by the different spacing and cultivars. However, the highest length of bulb (6.86 cm) was recorded from the variety Agrifound Light Red with spacing 15 × 10 cm followed by variety NHRDF Red-3 with 15x10cm cm (6.53 cm) and the lowest bulb length (4.86 cm) was recorded from the variety Agrifound Light Red with spacing 7.5 × 10 cm. Thus, the number of scale per bulb (8.33) was increased from the variety NHRDF L-28 with spacing 15 × 10 cm followed by variety Agrifound Light Red with spacing 15 × 10 cm (8.00). This is due to proper spacing ensures optimum growth and weight of bulb through adequate utilization of moisture, light, spacing and nutrients (Zubeldia and Gases, 1977 Habtamu et al., 2016). These results are conformity with (Kumar *et al.,* 1998), the highest yield with a spacing of 15 × 10 cm, (Kantona *et al.,* 2003) and (Khan *et al., 2003*) total bulb yield can be increased as population density increase and (Gupta and Gaffer, 1980) bulb size and bulb weight decreased with the decrease in spacing (Kishor *et al.,* 2017).

Table 1 indicated that interaction of spacing and different cultivars had influenced significantly on total soluble solids and ascorbic acid of onion bulb. The maximum total soluble solids (13.96^{0}Brix) were obtained from variety NHRDF Red-3 with spacing 10 × 10 cm followed by variety NHRDF L-28 with spacing 7.5 × 10 cm (13.50^{0}Brix) and minimum (10.66^{0}Brix) was recorded from variety Agrifound Light Red with spacing 7.5 × 10 cm. While ascorbic acid (9.93 mg/100g) was increased from variety NHRDF RED-3 with spacing 15 × 10 cm followed by variety NHRDF RED-3 with spacing 12.5 × 10 cm (9.20 mg/100g) and minimum (7.43 mg/100g) amount of ascorbic acid was recorded from variety Agrifound Light Red with spacing 7.5 × 10 cm.

Thus reducing sugar, non reducing sugar and total sugars were not significantly influenced by the different spacing and cultivars interaction. However, the maximum reducing sugar (8.50%) was recorded from variety NHRDF L-28 with spacing 15 × 10 cm followed by variety NHRDF Red-3 with spacing 15 × 10 cm (8.23%) and minimum (5.50%) was recorded from variety Agrifound Light Red with spacing 7.5 × 10 cm. While maximum non reducing (9.70%) and total sugars (17.70%) were recorded from variety Agrifound Light Red with spacing 15 × 10 cm followed by spacing 7.5 × 10 cm and 15 × 10 cm with variety

Table 1. Influence of different spacing and cultivars on yield components and biochemical parameters f Onion

Varieties	Spacing	Yield (kg/plot)	Yield (q/ha)	Weight of bulb (g)	Bulb diameter (cm)	Length of bulb (cm)	Number of scale per bulb	Total soluble solids (^{0}Brix)	Ascorbic acid (mg/100g)	Reducing sugar (%)	Non reducing sugar (%)	Total sugars (%)
Agrifoun Light Red	(7.5x10cm)	6.59	439.55	33.22	5.01	4.86	7.00	10.66	7.43	5.50	8.47	13.97
Agrifoun Light Red	(10x10cm)	7.34	489.77	36.00	5.05	5.40	7.00	10.76	8.60	6.63	8.37	15.00
Agrifoun Light Red	(12.5x10cm)	4.67	311.44	22.56	5.16	5.08	7.33	12.33	8.90	6.27	8.67	14.93
Agrifoun Light Red	(15x10cm)	5.81	387.33	38.20	6.86	6.86	8.00	12.53	8.83	7.93	9.77	17.70
NHRDF Red-3	(7.5x10cm)	6.88	458.77	44.33	5.49	5.40	7.00	12.50	8.83	7.73	8.83	16.57
NHRDF Red-3	(10x10cm)	6.47	431.77	40.36	5.48	5.36	7.33	13.96	8.43	7.37	8.67	16.03
NHRDF Red-3	(12.5x10cm)	5.83	388.88	48.13	5.68	5.03	7.33	12.76	9.20	7.83	8.57	16.40
NHRDF Red-3	(15x10cm)	6.71	447.33	55.43	7.06	6.53	7.66	12.06	9.93	8.23	8.67	16.90
NHRDF L-28	(7.5x10cm)	5.40	360.22	43.63	5.80	5.38	7.00	13.50	9.13	7.13	9.60	16.73
NHRDF L-28	(10x10cm)	6.14	409.55	49.80	6.52	5.10	7.33	13.23	8.40	7.60	9.00	16.60
NHRDF L-28	(12.5x10cm)	5.80	387.11	57.60	6.14	5.59	7.33	12.26	8.76	7.63	9.07	16.70
NHRDF L-28	(15x10cm)	5.66	377.77	55.00	7.00	6.51	8.33	11.86	10.03	8.50	8.97	17.47
	CD (P=0.05)	0.19	12.86	8.54	0.48	N/S	N/S	0.80	0.82	N/S	N/S	N/S
	SE(m)±	0.06	4.35	2.89	0.16	0.23	0.37	0.27	0.27	0.56	0.38	0.67

NHRDF L-28. This result is in agreement with the findings of (Gupta and Gaffer 1980), (Khan *et al.,* 2003) in onion and (Kumar *et al.,* 1998) obtained the better quality with spacing of 15x10 cm in onion.

CONCLUSION

Our study was concerted to the combined application of different spacing and varieties favorably influenced plant growth attributes. Results clearly emphasized the importance of spacing as well as selection of varieties of onion, as the conjoint use of them yielded higher and gave a remunerative return. Based on the trend of yield and economical aspects of onion observed in the present study; it was concluded that for getting higher bulb yield of onion, combined application of 10 × 10 cm spacing with var. Agrifound Light Red, was best under Lucknow conditions by 7.5 × 10 cm spacing with var. NHRDF Red-2 and 15 × 10 cm spacing with var. NHRDF Red-2. These results however need to be further confirmed on multi locations large scale trials before passing as recommendations to the onion growers of Lucknow.

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Investigations on the physical activity level of some Iranian drug suicidal patients

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ABSTRACT

As it is important to use the preventive and therapeutic effects of exercise and physical activity in the management of suicidal patients, we primarily need to know physical activity level of suicidal patients. However there are little data in this topic. The females to male ratio of patients were about 2.1:1. with the average age of 29.7, minimum 13 and maximum 88 years, with standard deviation of 12.1. Physical activity level of majority (76.4 percent) of patients was in the range of low (less than 600 Metabolic equivalent/minute per week). Other variables except sex were not associated significantly with the physical activity level of patients. Physical activity level was significantly lower in females than males (P = 0.001, Chi square test). Low physical activity level and female sex are possible risk factors for drug suicidal attempts in Iranian patients.

KEY WORDS: EXERCISE, MENTAL HEALTH, PHYSICAL ACTIVITY, POISONING, SUICIDE

INTRODUCTION

Suicide is a global public health problem. Annually, almost 1 million people die in suicides worldwide (Turecki et al. 2015). The global suicide rate in 2008 was about 11.6 per 100,000. However, suicide has an increasing incidence and it is estimated to be for more than 2% of the global burden of disease by the year 2020 (Behmanehsh Poor et al. 2014). Demographic, social, and cultural factors can affect suicidal epidemiology (Mirhashemi et al. 2016). As suicide is condemned in Islam, the suicidal rate is low in most Islamic countries,

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yet it has shown to have increasing rate in recent years (Pritchard et al. 2007). According to the last WHO report, the reported suicide rates in Iran per 100,000 was 5.3 in both sexes: 3.6 in females and 7.0 in males (Mirhashemi et al. 2016) but figure nearly 2 times greater for average suicide rate (9.9 per 100,000) was measured based on data elucidation (Hassanian-Moghaddam et al. 2017). Suicide and attempted suicide rates in Iran increased from 8.3 per 100,000 in 2001 to 19.4 in 2005, then declined to 16.3 in 2007 (Saberi-Zafaghandi et al. 2012). However, the officially reported figures for suicide are lower than what really occurred (Malakouti et al. 2015). Suicide with drug and self-immolation are two most common reported methods of suicide in Iran (Nazarzadeh et al. 2013, Poorolajal et al. 2015).

Physical training and an active lifestyle have been used as major public health tools in the prevention and treatment of many physical diseases including cardiovascular and metabolic diseases (Peluso et al. 2005). Also, the effects of exercise on the brain, cognitive function, and behavior have been of interest (Deslandes 2014). Based on several epidemiological studies, it has been shown that exercise and physical activity can have preventive or delaying effects on the onset of different mental disorders, and also can be used as therapeutic tools solely or in adjunct with other treatments for mental disorders (Zschucke et al. 2013). About 90% of individuals who had successful suicide had an identifiable psychiatric disorder before death (Arsenault-Lapierre et al. 2004) and at least 50% of suicide deaths are related to depressive episodes, either as major depressive disorder or bipolar disorder (Holma et al. 2014). In a Cochrane review which compared exercise intervention with no treatment, or with standard interventions (cognitive therapy and pharmacotherapy) in the management of depressive symptoms, a moderate clinical effect was found for exercise, which indicated that exercise was as effective as these standard treatments (Rimer et al. 2012). In a recent cross-sectional study which examined the relationship between suicidal thoughts (ST) and suicidal attempts (SA) and the level of physical activity (PA) among South Korean adolescents, there was an inverse dose-response relationship between the level of physical activity (defined as vigorous, moderate, and low) and ST and SA (Cho 2014). Also, in urban areas of the Iran, suicide is more common than rural areas (Shirazi et al. 2012). The cause of higher rate of suicide in cities may be related to lifestyle factors including more stress and lower physical activity level which can be the result of air pollution (Hajian et al. 2015).

Hajain et al. (2015) compared with those in rural areas. As it is important to use the preventive and therapeutic effects of exercise and physical activity in the management of suicidal patients, we primarily need to know physical activity level of suicidal patients in Iran. There is no data on the physical activity level of suicidal patients in Iran. So, the aim of the present study was to investigate physical activity level of drug suicidal patients in a referral hospital of poisoning management in Tehran.

MATERIAL AND METHODS

In the current cross-sectional study, the Persian-translated long form interview-administered International Physical Activity Questionnaire (IPAQ) was used for assessing physical activity level of 406 drug suicidal patients who were admitted to poisoning ward of Loghman Hospital (the next morning after admission) between December 2014-April 2015. The validity and reliability of this version of IPAQ have already been proven in Iranian sample of individuals (Vasheghani-Farahani et al. 2011). After obtaining the consent form, interview was conducted by an expert with alive drug suicidal patients who had good consciousness level and cooperation or with close relatives of the patients who were informed about the physical activity habits of the patients. The results were expressed as low (less than 600 metabolic equivalent (MET). minute per week), medium (between 600 to 3000 MET. minute per week), and high (more than 3000 MET. minute per week) level of physical activity. Also, the patients were asked about their age, weight, marital and educational statuses, number of children, absence or presence, and in this case, the type of background mental disorders, the history and the number of previous suicide attempt(s). The data was then analyzed using SPSS (v. 16) and related statistical tests (ANOVA test for quantitative and Chi square test for qualitative variables).

RESULTS

Totally, 406 patients were evaluated: 278 females (68.4%) and 128 males (31.6%) with the average age of 29.7, minimum 13 and maximum 88 years, with standard deviation of 12.1. The average weight of patients was 64.8 kilograms, minimum 37 and maximum 145 kilograms, with standard deviation of 15.6. Other epidemiologic characteristics of patients are provided in Table 1. A total of 310 patients (76.4%) had low physical activity level, 80 patients (19.7%) medium, and 16 patients (3.9%) had high physical activity level. There were no significant differences in weight, number of children, and suicide history, marriage and educational status between patients with different physical activity levels (p value more than 0.05). There was significant difference in terms of different physical activity levels between the two sexes (p value = 0.001), which means females in low

Table 1. Demographic properties of drug suicidal patients

Variable	Description	Number	Percent
Marriage status	Single	187	46.1
	Married	202	49.8
	Divorce	17	4.2
Number of children	0-1	320	79
	2-3	71	17.5
	More than 3	15	3.5
Education	Up to Diploma	305	75.1
	Diploma to BSc	86	21.1
	MSc and higher	15	3.6
Mental disorder (according to patient's statement)	No disease	317	78.1
	Depression	52	12.8
	Anxiety	3	.7
	Psychosis	2	.5
	Bipolar	4	1.0
	Other	20	4.9
	0	245	60.3
History of suicide	1	70	17.2
	2	15	3.7
	3	14	3.4
	4	5	1.2
	5	4	1.0
	6	3	.7
	7	1	.2

physical activity group had relatively higher percentage than their male counterparts and males in moderate and high physical activity groups had relatively higher percentage than their female peers.(Table 2).

DISCUSSION

According to the findings of the present study, from among 406 drug suicidal patients admitted to Loghman Hospital, the majority (76.4% percent) had low physical activity level. In a randomized cross over trial in high-level suicide risk patients, regular endurance training in the form of mountain hiking was shown to reduce hopelessness, depression, and suicide ideation (Sturm et al. 2012). Moreover, the moderate level of evidence has been reported for the inverse dose-response relationship between mental disorders (depression and distress) and physical activity level in the latest guideline for exercise testing and prescription of American College of Sports Medicine (ACSM) (Ferguson 2014).

Table 2. Physical activity levels of drug suicidal patients according to sex of them

			Physical activity Level			Total
			low	moderate	high	
Sex	*female*		224	49	5	278
		Percent	80.6%	17.6%	1.8%	100%
	male		86	31	11	128
		Percent	67.1%	24.2%	8.7%	100%
Total			310	80	16	406
		Percent	76.4%	19.7%	3.9%	100.0%

The mechanisms whereby exercise and physical activity induce positive psychological health are diverse and complex. Broadly, it can be divided into reducing stress response, minimizing excessive inflammation, and enhancing growth factor expression and neural plasticity. Physical fitness, achieved through regular exercise and/or spontaneous physical activity blunts stress reactivity, protects against potentially adverse behavioral and metabolic consequences of stressful events of life (Silverman et al. 2014).

Physical activity level of the patients of the preset study was measured by IPAQ includes both physical activity and exercise, i.e. a type of physical activity that is planned, structured, repetitive, and purposeful (Carek et al. 2011). Although much of the research on positive psychological effects of exercise has been done on aerobic exercise, resistance exercise or strength training can also induce many physiological and psychological advantages. Increase in cognition and self-esteem, and decrease in depression and anxiety after both single-bout sessions and long-term resistance training have been identified in growing body of literature (Strickland et al. 2014). Also there are evidences that indicate that outdoor physical activity interventions such as mountain hiking, stimulates higher positive and lower negative affective responses compared to an indoor physical activity condition(Niedermeier et al. 2017).

In our study, the majority (68.4 percent) of drug suicidal patients were female. Also, the female to male ratio was higher in low physical activity level group. These sexual differences have been seen in the previous studies (Esteghamati et al. 2011, Koohpayehzadeh et al. 2014), as well, and may be due to Iranian cultural and social backgrounds which encourage men to be more physically active compared with women.

The most common underlying mental disorder in our study was depression. Depression was also found in other studies as a risk factor for suicide and suicidal thought and self-destructive behavior in Iranian people (Ekramzadeh et al. 2012, Dabaghzadeh et al. 2015). It has been reported that the risk of suicide is increased by more than 50 percent in depressed individuals and it is about 20 times that of the general population in people

with major depression (Baek et al. 2015). There is now compelling evidence that lifestyle factors including diet, physical activity and exercise, relaxation and meditative techniques, quality of sleep, environmental pollutants, and social support are significant in the pathogenesis and treatment of depression; moreover, epidemiological studies have shown that low physical activity can be a risk factor for the development of depressive symptoms and that regular physical activity in early years of life is linked with reduced risk of experiencing depression in adulthood (Sarris et al. 2014).

Therefore, prevention of depression and other mental disorders by doing regular physical activity and exercise is a cost-effective approach for people who experience higher rates of mental illness compared with the general population. Indeed, mandatory physical exercise for the prevention of mental illness has been proposed for medical students in USA (Bitonte et al. 2014) as high prevalence of depression and anxiety has been reported among USA and Canada medical students, with levels of overall psychological distress indicators consistently higher than that in the general population and age-matched peers (Dyrbye et al. 2006). This approach can be used in the public suicide prevention programs too, as exercise has been shown to improve stress management ability, general feelings of well-being, and self-esteem and can act as a nonspecific psychological therapy (Kaminsky et al. 2006).

CONCLUSION

Low physical activity level and female sex are possible risk factors for drug suicidal attempts in Iranian patients. More precisely designed studies are needed to evaluate the relationship between physical activity and suicidal attempts in females. Promotion of increasing physical activity and exercise for patients at risk of suicide especially females, are recommended.

LIMITATIONS

The study was done in a drug poisoning management center and suicidal patients with causes other than drug poisoning were not included. Also the cases were alive patients and the dead ones were not included.

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CONFLICT OF INTEREST

There is no conflict of interest.

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Bond strength of porcelain to cobalt chromium dental alloy fabricated by selective laser melting and casting methods

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ABSTRACT

This study aimed to compare the bond strength of porcelain to cobalt-chromium dental alloy fabricatedby selective laser melting (SLM) and casting methods.Twelve rectangular barsmeasuring 25x3x0.5 mmwere fabricated of cobalt-chromium alloy for each of the SLM and casting groupsaccording to ISO9693:1999. Porcelain was appliedat the center of each bar measuring 3 × 8 mm with 1 mm thickness. Three-point flexural bond strength test was performed to assess the bond strength of porcelain to alloy. Data were analyzed and compared between the SLM and casting groups via Independent sample t-test (alpha=0.05). Mode of failure was also determined by visual inspection of samples. The mean bond strength of porcelain to SLM alloy (35.26±1.22 MPa) was significantly higher than that to casting alloy (33.21±3.02 MPa) ($P<0.05$). Most failures in both groups were mixed and no sample showed adhesive failure. The SLM metal-ceramic system showed higher bond strength than the required threshold by ISO9693:1999. Compared to alloys fabricated by the casting method, the SLM method showed higher bond strength to porcelain. This relatively new technology is promising for dental application and can serve as a suitable alternative to the conventional casting method for the fabrication of metal-ceramic restorations.

KEY WORDS: SHEAR STRENGTH; METAL-CERAMIC; LASER MELTING

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INTRODUCTION

Metal-ceramic systems for dental restorations are available since the 1960sand the success of metal-ceramic restorations depends on the strength and uniformity of bond at the metal-porcelain interface, which is the most sensitive area in terms of crack formation (Bowers 1985) and (Drummond and et al 1984, Pavlović 2017 and Abduo 2017). Cracks mainly occur at the metal-ceramic interface or within the veneering porcelain (Daftary 1986) and (Zhukovsky 1996). Factors such as trauma, fatigue, occlusal loads and incompatibility between the mechanical properties of metal and porcelain may result in porcelain fracture especially of cohesive type (Pameijer 1996 and Kelsey 2000,Ren 2016 and Kaleli 2017). Metal-ceramic frameworks are conventionally fabricated using the lost wax casting (Anusavice 2003) and (Kaleli 2017).

However, problems associated with casting of base metal alloys such as high melting point, high oxidation potential during casting, time consuming nature of casting and finishing of these alloys, limit their application. Thus, application of a technique to eliminate the casting process may enhance the use of these alloys (Bhaskaran 2013) and (Akova 2008).

The selective laser melting (SLM) technology, also known as the three-dimensional (3D) printing, was introduced for the fabrication of metal copings in metal-ceramic crowns. This technique does not have many of the limitations of the conventional waxing technique, so thatthe restorations are fabricated by incremental application of 0.02 µ layers (Xin 2012). This technique works based on the computer aided design (CAD) data obtained from the framework design, and uses high temperature laser beams for selective melting of metal framework, which is in the form of powder. The metal framework is formed by incremental addition of these thin layers (Akova 2008).

The success of SLM is related to its ability in fabrication of metal components with complex geometrical shapes from a 3D CAD model. Moreover, this technique can produce components with mechanical properties comparable or superior to those of conventionally fabricated components (Örtorp 2011) and (Quante 2008).Thus, it can be an alternative to the conventional process. Only a few studies have assessed the bond strength of porcelain to alloys fabricated by the SLM technique. Some of these studies have reported superior mechanical properties for copings fabricated by this method and higher bond strength of porcelain compared to those fabricated by the conventional casting method ,while some others found no significant difference in bond strength of porcelain between the two groups (Liu YH 2010) and (Wu 2014) and (Ren 2016).

Considering the gap of information and the controversies in this regard, this study aimed to assess the bond strength of cobalt-chromium (CoCr) alloy fabricated by the SLM technique and the conventional wax burnout and casting method.

MATERIALS AND METHODS

This in vitro, experimental study was conducted on 24 samples (12 fabricated by conventional casting and 12 fabricated by the SLM technique). In the casting group,12rectangular bars were fabricated measuring 25 mm ± 1mm × 3 mm ± 0.1 mm × 0.5 mm ± 0.05 mmaccording to the ISO9693-1 (The International Organization for Standardization 2012) using green wax sheets (Berg, Karl Berg GmbH, Engen, Germany). They were spruedand flasked using phosphate-bonded investment material. After wax burnout at 650°C, melted CoCr was poured into the space created by wax burnout using a standard broken-arm centrifugal casting machine (Kerr, Orange, CA, USA). Metal sheets were cleaned from investment material and the sprues were cut. Dimensions were adjusted and the thickness of sheets was ensured by a caliper and gauge.

In the SLM group, 12 samples were fabricated with the same dimensions as those in the casting group using EOSINT M270 machine (Manufacturing system; Bego, Bremen, Germany).This device was equipped with ytterbium laser fiber with less than 200 W laser power, scanning speed of 5-10 mm/second, wavelength of 1060-1100 nm, beam diameter of 100-500 µm and powder feed rate of 5-7 g/minute. Using data obtained from a CAD file with STL format, the device fused metal particles and after scanning each cross-section, the alloy thickness decreased by one layer and a new layer of alloy was applied on the upper surface. This process was repeated until the entire restoration was formed. Table 1 shows the composition of CoCr alloy used for the casting (WBC 9581TM, Bego, Germany) and SLM (EOS Cobalt Chrome SP2; EOSGmbH, Germany) techniques.

The samples in both groups were then subjected to air-borne particle abrasion for five seconds (with 110-250 µ aluminum oxide particles at 3-4 MPa pressure for the SLM alloy and 120 µ aluminum oxide particles at 0.35 MPa pressure for the casting alloy) to provide surface roughness similar to the standard process practiced in the clinical setting. The samples were then cleaned with vapor spray for six seconds. Pre-oxidation was then performed according to the manufacturer's instructionsat 950-980°C for five minutes for SLM and 980°C for eight minutes for the casting alloy. Air-borne particle abrasion was then performed again and before porcelain application, the samples were cleaned in an ultrasonic

Table 1. Composition of CoCr alloy used for the casting and SLM techniques according to the manufacturers (wt%)

Group	Co	Cr	Mo	W	Si	Nb	V	Fe	Mn	Ce
SLM alloy	63.9	25.3	5.2	5.5	1	–	–	Max 0.5	Max 0.1	–
Cast alloy	61	26.6	5	<2	–	–	<2	–	–	<2

Co, cobalt; Cr, chromium; Mo, molybdenum; W, tungsten; Si, silicon; Nb, niobium; V, vanadium;Fe, iron; Mn, manganese; Ce ,Cerium; SLM, selective laser melting; max, maximum.

bath containing 95% ethanol (Elmasonic, Elma Hans GmbH & Co, Koln, Germany) for five minutes.

Next, equal layers of opaque and body porcelain (VMK 95, Vita, Bad Säckingen, Germany) with 8 mm length, 3 mm width and 1 mm thickness (0.2 mm opaque porcelain and 0.8 mm body porcelain) were applied and baked according to the manufacturer's instructions (Figures 1 and 2). For the purpose of standardization of porcelain thickness, a silicon index was used for all samples (Figure 3). To minimize the effect of confounders, all procedures were performed by one operator.

A three-point flexural strength test was performed according to the ISO9693-1 standard using a universal testing machine (Z020, Zwick Roell, Germany) .Surface of the sample with porcelain over it was placed downward and the two ends of each rod were placed on the supporting fulcrums with 0.9 mm diameter and 20 mm distance from each other. Load was applied to the center of each sample by a crosshead with 0.9 mm diameter at a speed of 1 mm/minute(figure 4). Load application was continued until failure (abrupt drop in the stress-strain curve). The load at failure was recorded and reported by the computer. Bond strength in megapascals (MPa) was calculated using the formula A= k x F (N/mm2) where k

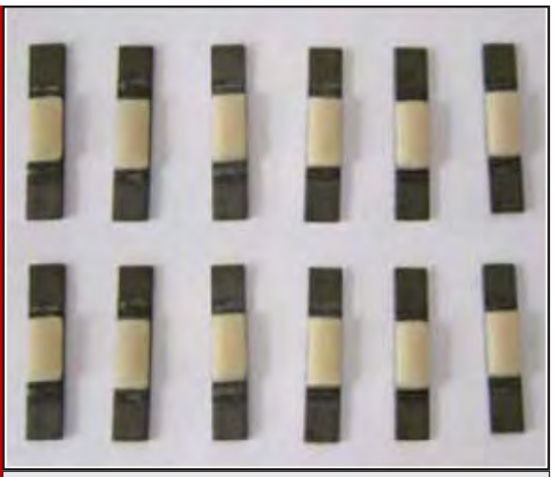

FIGURE 1. Casting group samples

FIGURE 2. SLM group samples

FIGURE 3. Silicon index used for porcelain application

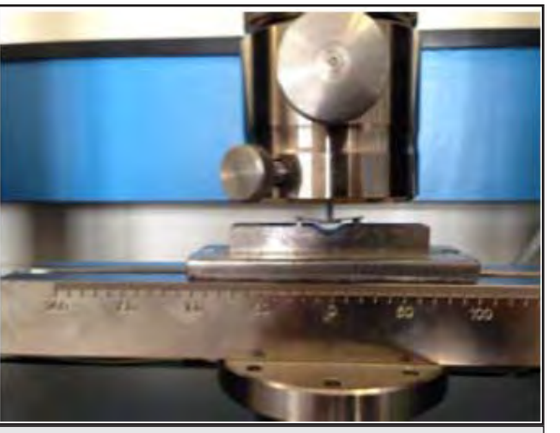

FIGURE 4. Three-point flexural test

Table 2. Mean and standard deviation of bond strength in the two groups (MPa)

Group	Mean± standard deviation
SLM	35.26 ±1.22
Casting	33.21 ± 3.02

is a constant related to thickness and modulus of elasticity of the alloy and F is the maximum load in Newtons causing debonding.

After conduction of flexural test, the samples were visually inspected to determine the mode of failure. Mode of failure was divided into three groups of adhesive(at the metal-porcelaininterface), cohesive (completely within the porcelain) and mixed (a combination of adhesive and cohesive types).

Finally, Data were analyzed by Independent samples t-test.

RESULTS AND DISCUSSION

Table 2 shows the mean and standard deviation of bond strength in the two groups.

Independent samples t-test was used to compare the mean values between the two groups, which showed that the mean bond strength of porcelain was significantly higher to alloy in SLM group compared to the casting group (p=0.04).

Inspection of the surface of samples in the SLM group before porcelain applying showed that the surface of bars in the SLM group was dark gray without metal shine. Figure 5 shows photography of the surface morphology of metal matrix of the one sample of each group after porcelain debonding. Visual inspection of each sample during flexural strength testing showed that debonding cracks between metal and ceramic occurred at one end of the ceramic layer and not at the center.

Table 3. Mode of failure in the two groups

Group	Adhesive failure	Cohesive failure	Mixed failure
SLM	0	5	7
Casting	0	0	12

In terms of mode of failure, in the SLM group, most failures were mixed and no adhesive failure was noted. In the casting group, all failures were mixed (Table 3).

DISCUSSION

Failure of the metal-porcelain bond can occur as the result of a combination of factors such as different coefficients of thermal expansion of metal and ceramic, presence of micro-cracks within the porcelain and occlusal loads or trauma. Loss of porcelain to metal bond is the second most common cause of replacement of metal-ceramic restorations [18]. The reason for use of SLM technology in this study was the recent introduction of this technique and its numerous advantages. The SLM technique enables the fabrication of restorations with a more uniform quality and standardization of restoration shaping process with lower cost, requiring less human force, saving time due to elimination of many procedural steps (compared to the conventional method) and elimination of human errors. Moreover, CAD, compared to manual waxing, results in more accurate control of the porcelain thickness and decreased risk of fracture. SLM scanners have disadvantages as well including limitation of scanning systems in terms of resolution and creating rounder margins as well as creation of cloudy spots caused by scanning, which result in internal mismatch (Walton 1986) and (Huang 2015) and (Li 2014). High cost of laser device used in this technique is another disadvantage of this method.Also, the CoCr alloy

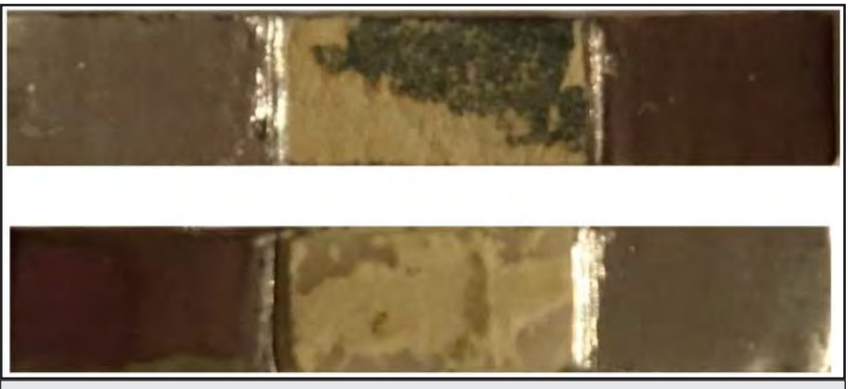

FIGURE 5. the surface morphology of metal matrix in casting sample(the upper) and in SLM sample (the lower)

fabricated by the SLM method has not shown any cytotoxic potential and evidence shows that ithas higher corrosion resistance and lower release of cobalt ion compared to the casting group (Xin 2012).

Among the suggested tests for assessment of bond strength, Schwickerath crack initiation test or three-point flexural strength test was first suggested by Lenz et al, and is alsorecommended by the ISO9693:1999(E) for determination of debonding strength of metal-ceramic systems(Lenz 1995). Although several mechanical tests such as flexural, torsional, shear, tensile or a combination of flexural and shear tests can be used for assessment of the bond strength at the metal-ceramic interface, the validity of the bond strength assessment tests is still questionable (Kontonasaki 2008).

The three-point flexural strength test reveals the modulus of elasticity, flexural strain, flexural stress and flexural stress-strain response of a material. The main advantage of this test is easy sample preparation and conduction. However, it has drawbacks as well. One drawback is that the test result is affected by the geometry of samples, load application and speed of strain application (Kontonasaki 2008). For this reason, in the current study, we tried our best to control for all the confounding factors that had the potential to affect the results such as speed of load application (crosshead speed), distance between the two fulcrums and dimensions of the samples. All samples were fabricated with the same dimensions. Also, a custom-made silicon index was used for control of the length and thickness of ceramic layer when applying the porcelain.

In order to acceptably compare the bond strength of ceramic to metal between restorations fabricate by two different techniques, the materials used should be the same in terms of behavior with regard to the formation of oxide layer, coefficient of thermal expansion, etc. as much as possible (Kontonasaki 2008) and (Zinelis 2003). Therefore, in the current study, alloys with the most similar composition were chosen for SLM and casting groups in order to minimize the effect of elements in the composition of alloys and assess the pure effect of fabrication method on the results .

The bond between the porcelain and metal can be achieved bythree mechanisms of van der Waals forces, mechanical retention and chemical bond; chemical bond is the dominant factor responsible for metal-porcelain bond. Chemical bond is influenced by the composition of elements in the alloy and formation of oxide layer on the metal surface. Due to the significance of the thickness of oxide layer in a successful bond, the pre-oxidation phase of alloy was performed for both groups in our study prior to the addition of porcelainaccording to the manufacturer's instructions (Bagby 1990) and (Jochen 1986).

In this study, the SLM group showed higher porcelain bond strength compared to the casting group, which was in line with the results of Liu et al [14]. One possible reason for higher bond strength of the SLM group was the surface properties of this alloy. The SLM system works based on incremental application of melted powders. The surfaces of fabricated components by the SLM technique often have sticky powder. In other words, relatively melted powders are added to the surface of components and make them rougher (Zhang 2014). Due to the inherent roughness of the surface ofSLM samples and according to the results of a study by Zhang (Zhang 2014), it may be stated that these surfaces can increase the contact area of framework and porcelain. At the same time, surface porosities can provide strong micromechanical retention for porcelain and enhance the bond strength (Zhang 2014).

Another possible explanation is penetration of porcelain into the gaps created between layers as well as the balling phenomenon during incremental fabrication by the laser sintering process, which increase the contact area and bond strength by creation of undercuts (Eun-Jeong 2014) and (Gu D 2007).

The fact that all tested samples in our study yielded higher bond strength than the minimum strength required by ISO9693:1999(E), which is 25 MPs shows that the alloy fabricated by the SLM technique can not only provide an acceptable bond for clinical application, it even yields higher bond strength than the casting method. Akova and Serra-Prat in separate studies showed that despite higher bond strength of the casting group, the difference between the casting and SLM techniques did not reach statistical significance (Serra-Prat 2014). Controversy between their results and ours may be attributed to the different form of samples (cylindrical) and assessment of shear bond strength instead of flexural bond strength.

Xiang and Ren found no significant difference in bond strength of the SLM and casting groups; although the values obtained in the SLM group were higher in both studies (Xiang 2012). Difference between their results and ours may be due to difference in the load application speed for flexural test, difference in laser system and composition of alloys used and size of sand-blasting particles.

Inspection of the surface of samples in the SLM group before porcelain applying revealed that the surface of bars in the SLM group was dark gray and had no metal shine in our study. The reason may be that the metal powder particles are fused on the surface of components fabricated by the SLM method; thus, the samples show a color similar to that of metal powder particles.

Visual observation of each sample during the flexural strength testing revealed that the debonding cracks at

the metal-ceramic interface occurred at one end of the ceramic layer and not at the center of it, which was in agreement with ISO9693:1999(E) standards. Evaluation of surface morphology of metal matrix after porcelain debonding showed that the color was different at the center and at the ends of the samples. This indicated that the ceramic layer was still present on the entire surface of samples. Moreover, mode of metal-ceramic debonding may indicate the fracture energy that degrades the metal-ceramic bond. The higher the amount of porcelain body remaining on the metal surface, the higher the adhesion of porcelain body to metal, the higher the fracture energy and the lower the risk of porcelain fracture would be in the clinical setting (Wagner 1993) and (Lavine 1966). Five samples in the SLM group showed cohesive failure while in the casting group, all fractures were mixed. The reason is probably the higher bond strength of the SLM group. Since after debonding high amounts of porcelain remained on the alloy surface, it may be concluded that a strong bond was created between dental porcelain and SLM alloy at the interface. Thus, this technique can increase the success rate of porcelain fused to metal restorations.

Since this study had an in vitro design, the SLM alloy should also be evaluated in the oral cavity. Also, it is recommended to assess the marginal fit of copings fabricated by this technique. The bonding interface should be evaluated using scanning electron microscope and energy-dispersive X-ray spectroscopy in future studies. The microstructure of SLM alloy must also be analyzed under a metallographic microscope.

CONCLUSION

The SLM alloy system not only provides a clinically acceptable bondstrength , the bond strength of restorations fabricated by this method to porcelain was even higher than that of restorations fabricated by the conventional casting method. Thus, this relatively new technology can serve as an alternative to the conventional casting method for the fabrication of metal-ceramic restorations.

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Spatial and temporal ground water responses to seasonal rainfall replenishment in an alluvial aquifer

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ABSTRACT

Groundwater is an enormously vital water resource available on earth. Due to over exploitation of groundwater, especially in dry regions and in the water scares areas, has led to irretrievable consequences due to deterioration of water quality. The groundwater resources, which are found in shallow depth often have poor in water quality and in most cases the ground water are severely over-exploited. Therefore, the study has been planned to identify the spatial & temporal variation of aquifer responses of the study area to the natural recharge as well as Ground water level trend in last 15 years. This study is conducted in Tawa command area, which lies in Hoshangabad District of Madhya Pradesh. Also facing problem of water logging and shortage in the region. The study identified the spatial behavior of ground water level in pre-monsoon and post monsoon. Insight of the current study, the spatial and temporal graphical analysis for the observation sites of the unconfined aquifer indicated that there is a good hydraulic connection of groundwater level with the rainfall.

KEY WORDS: AQUIFER, GROUNDWATER RESOURCES, WATER LOGGING, WATER LEVEL FLUCTUATION

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INTRODUCTION

Water is the most vital resource of both biosphere and human society, without which no life can sustain on the earth. Over 95% of the earth's useable freshwater is stored as groundwater, of which about 50% exists within the earth's crust down to a depth of 800 m, while only 1.5% exists in rivers and lakes (World Bank, 2010). Over exploitation of groundwater for irrigation and other purposes, especially in dry climates and in areas which are facing water stress, has led to irreversible consequences. The water stress on different sessions leads to Groundwater-level fluctuations, which basically represent hydraulic responses to changes in groundwater storage due to aquifer recharge and drainage as well as to changes in stress that include water mass loading and unloading above the aquifer surface (Burgess et al., 2017).

In order to meet the requirement of fast growing population and industrialization, the water demand has been increased significantly all over the globe. Overexploitation of groundwater in many part of the world has resulting a threatening condition of falling groundwater levels, saline water infringement, crop demand-supply gap, drying of the shallow aquifers, increasing power consumption due to increase in cost of lift, decrease in free flow and even local subsidence in some places (Singh and Singh, 2002).

In many regions there is a lack of surface water resource and the water bearing sub-surface zones are having severe water contamination. Shallow groundwater resources are often of poor quality and above that they are severely over-exploited. In a recent study based on the analysis of GRACE satellite data revealed that the groundwater resources in the states of Rajasthan, Punjab and Haryana are being depleted at a mean rate of mean rate of 4.0+1 cm yr-1 (Rodell et al., 2009).

Thus, it is evident that the current patterns of water development and consumption are not sustainable in several countries of the world, including India. Impending global/regional climate change, increasing population and growing socio economic changes are expected to exacerbate existing groundwater depletion through considerable rise in water demand (Sauer et al., 2010; Brown et al., 2013) and alterations in the spatio-temporal availability of freshwater resources (Healy, 2002; Elliott et al., 2014; Schewe et al., 2014; Bouwer, 2002; CGWB, 2012; Singh, 1997; Kumar, 2002; Vanderzalm et al. 2015 Nema 2017, and Smith et al., 2016).

Therefore, there is an urgent need for efficient management of freshwater resources in India in order to ensure sustainable management of our depleting freshwater resources.In many parts of India, The water table is declining at a very fast rate. In few locations, it has been gone beyond to the depth where it is uneconomical for pumping water for non-commercial and commercial uses. In few states, the situation is so critical that Government has banned ground pumping to stabilize the ground water. Groundwater extraction rate is not only the one factor which is responsible for declining water levels, the low rainfall resulting due to inadequate monsoon is equally responsible. Majority of the ground water stress areas categorized as overexploited and critical units also lies in such states. Considering the importance of Groundwater, the issue of Ground water declination or waterlogging has to be taken with priority considering all the proved water management technologies along with efficient strategies shall be adopted that could help to reduce the prevailing disaster. This current study demonstrate a comprehensive analysis of the effects of spatial and temporal precipitation patterns on Ground water level fluctuations.

MATERIAL AND METHODS

Hoshangabad District which is situated in Upper Narmada Region, M.P., lndia (Fig.1) was selected for this study. Narmada River, the other main river of Hoshangabad is Tawa River, which flows towards north and joins river Narmada near Hoshangabad.District Hoshangabad covers majority of Tawa command areas which are the primary sources of irrigation for entire district. The Hoshangabad district lies between north latitudes 22° 15' and 23° 00' and east longitudes 77° 15' and 78° 42' in part of survey of India topo sheet Nos, 55F & 55J. Hoshangabad is the district headquarter and Sohagpur, Piparia, Babai, Pachmarhi, Seoni Malwa and Bankheri are some of the major towns. The location map of study area is represented in Fig. 1. Soils of the area are characterized by black grey, red and yellow colors, often mixed with red and black alluvium and ferruginous red ravel or lateritic soils.

Northern part of the study area (i.e. Hoshangabad district), adjoining the Narmada river is covered with alluvium, which makes for more than 50% of the entire district.

Deccan traps occur as lava flows in the west central part of the district. The southern part of the district is hilly and occupied by rocks belonging to Gondwanas. There are two aquifer systems present in the study area. The top phreatic aquifer range in thickness from 4 to 30m and is encountered in the depth range of 4 to 20 mbgl. The phreatic aquifer has intercalations of clay and silt, and at places also of coarse sand or gravel. The Deeper aquifer (i.e. confined aquifer) ranges varies from 30 m to 60 m.

WATER LEVEL FLUCTUATION IN THE STUDY AREA

Water table Declination in summer season is a common problem for many part of the state as well as for India. The current study has been done to observe the ground

FIGURE 1. Location map of the study area

water level of study area with observing the rainfall (pre monsoon and post monsoon) and river gauge impact over ground water level for 16 years period from year 2000 to year 2015. The fluctuations have been observed in relation to rainfall (pre monsoon and post monsoon). The water table fluctuations have been influenced by many factors. The decline of groundwater level occurs when the outflow exceeds or less recharge over the area. The groundwater-level level data of 74 observation wells tapping unconfined aquifer were collected for 15 years (2000-2015) from the Central Ground Water Board (CGWB) and the Ground Water Survey and Investigation (GWS&I), Bhopal, Madhya Pradesh.

RESULTS AND DISCUSSION

SEASONAL WATER LEVEL FLUCTUATIONS DUE TO RAINFALL (YEAR 2000 TO 2015)

Monthly variations of rainfall in the study area over 16-year period (2000-2015) at eight rainfall/rain gauge stations along with the line graphs are shown in Fig. 4.2 It is apparent from Fig. 2 that though the rainfall events are somewhat distributed throughout the year, however the magnitude of rainfall is quite less or zero in some places. The rainy season usually starts from mid-June and lasts up to the end of October.

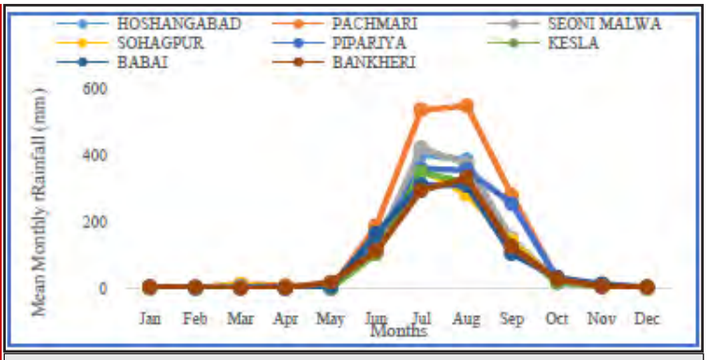

FIGURE 2. Monthly variations of rainfall in the study area over 16 years (2000-2015)

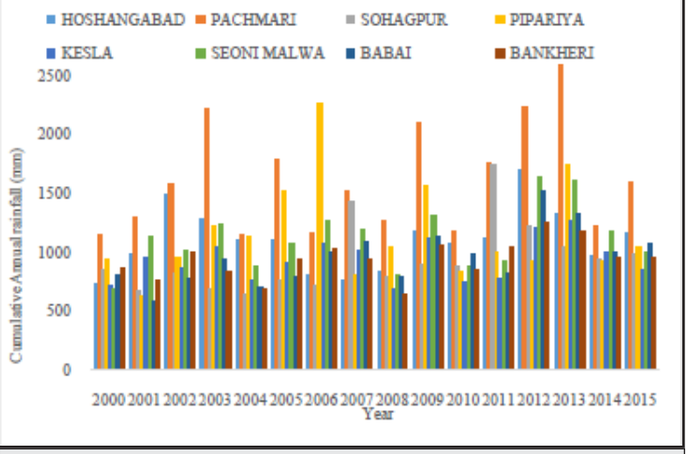

FIGURE 3. Variations of Annual Rainfall during 2000-2015 Period at Eight Rainfall Stations

Thus, the bulk of the annual rainfall is concentrated in a relatively short span of time and hence runoff and groundwater recharge are confined to these months. As sufficient rainfall is available during July, August, September and October, groundwater withdrawal is minimum during these months. The standard line graph indicate monthly variation of rainfall over 16-year period. The large standard line graph during May to October indicated considerable temporal variation of rainfall in these months (Fig. 2). However, during November through April, the variation in monthly rainfall is very small. Furthermore, the rainfall of Pachmari station has the highest rainfall for month of August, followed by Hoshangabad & Seoni Malwa.

Long-term variations in the annual rainfall of the study area from 2000 to 2015 are shown in Fig. 3 for the

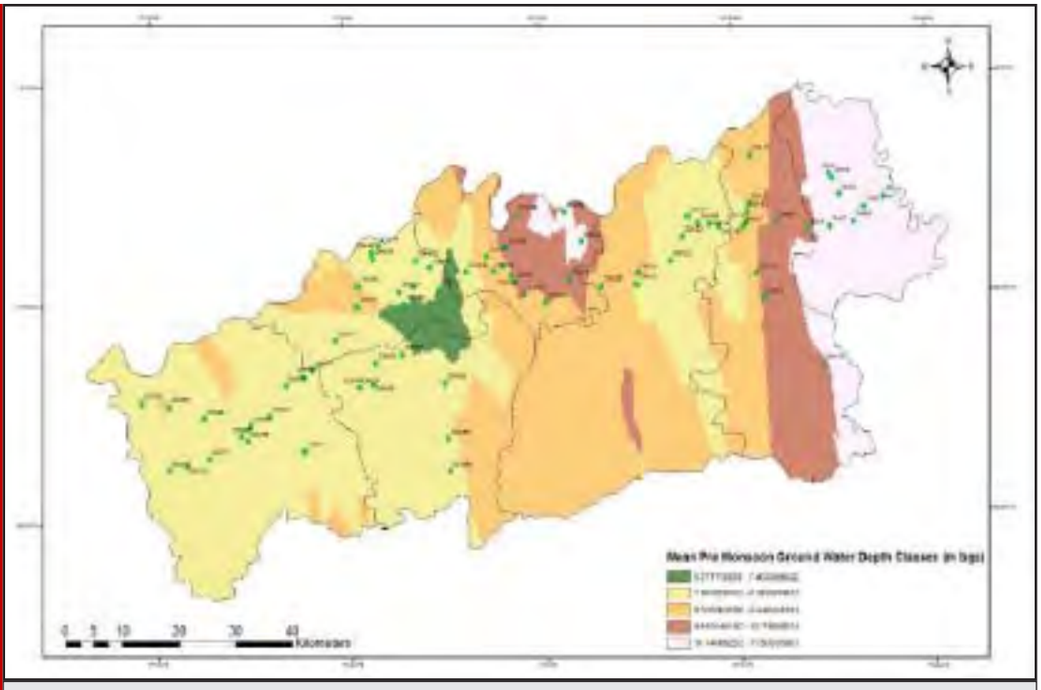

FIGURE 4. Spatial Variations of Mean Pre Monsoon Ground water Depth

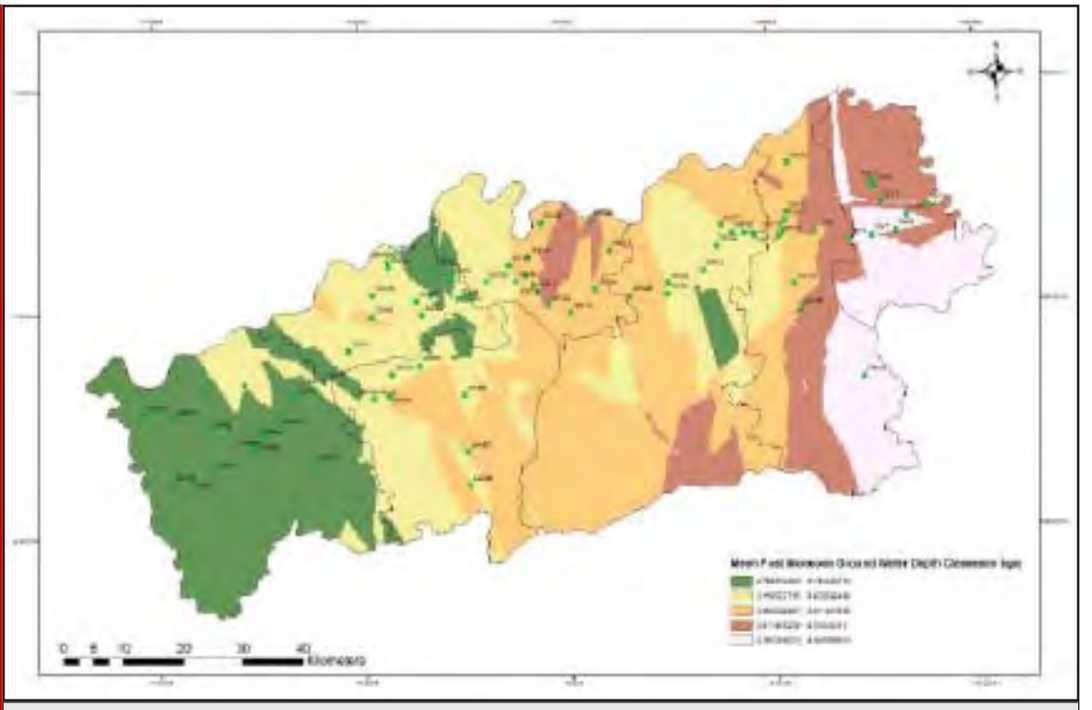

FIGURE 5. Spatial Variations of Mean Post Monsoon Ground water Depth

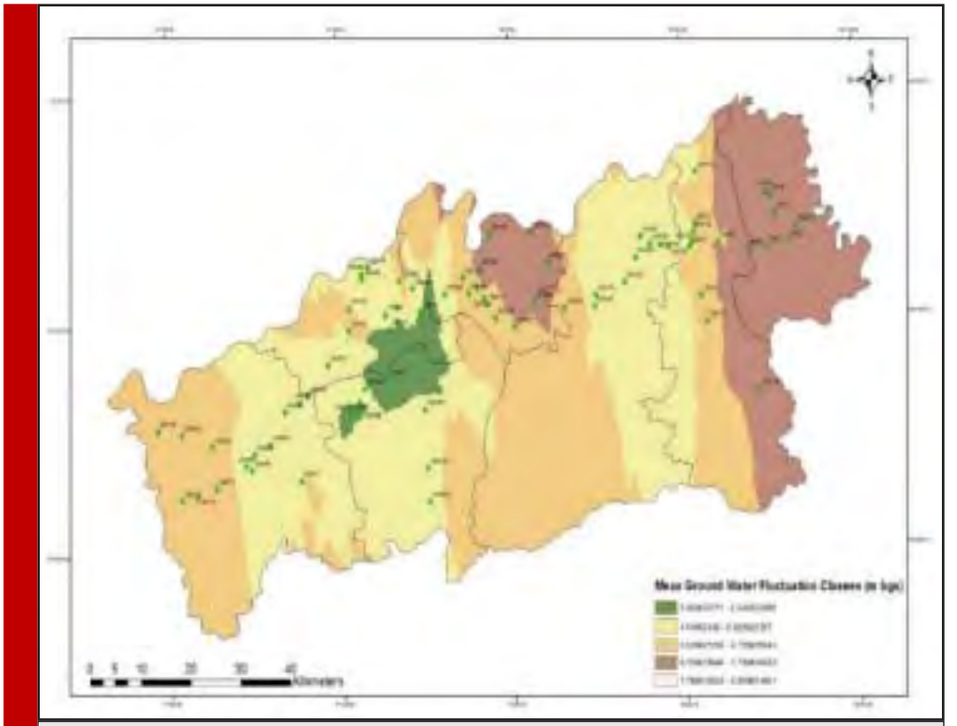

FIGURE 6. Spatial Variation of Mean ground water fluctuations depth classes

eight rain gauge stations. The average annual rainfall over the study area is estimated at about 1099.24 mm based on 16 years (2000-2015) rainfall records. In contrast, the rainfall at Pachmari station was found highest and found above than average annual rainfall for all years. The analysis were also done to find out the spatial variation of mean ground water fluctuation depth class (Mean of 2000-2015) over study area as shown in Fig.4., Fig. 5. & Fig. 6.

The minimum pre-post water fluctuation was observed in Hoshangabad and Kesla Block. The fluctuation of Pre-Post-monsoon groundwater depths (mean of 2000-2015) was maximum in Bankhedi followed by Babai, Sohagpur and Seoni Malwa block over the study area is shown in Fig. 5 & Fig. 6. It is clearly evident that the Bankhedi block of Hoshangabad showing the maximum water fluctuation depth which may be due to the non-command area exist on Bankhedi (Nema, 2017)

The average annual water level at downstream site varies from 5.5 to 7.0 m in last 15 years. The rainfall amount and ground water level from mean sea level has been found inversely proposal similar to upstream and middle stream sites.

FIGURE 7a. Temporal Response of Average annual ground water level with respect to Rainfall for upstream region

FIGURE 7b. Temporal Response of Average annual ground water level with respect to Rainfall for midstream region

FIGURE 7c. Temporal response of Average annual ground water level with respect to Rainfall for downstream region

The analysis of groundwater-depth contour maps of pre- and post-monsoon seasons revealed that the mean seasonal groundwater fluctuation in the study area ranges from 3.45 to 7.70 m, with a major portion of the study area having a mean groundwater fluctuation of 4.50 to 6.70 m.

Temporal analysis of Ground water level had been done in to upstream, mid stram and down stram sites represented in Fig 7(a-c). It has been evident from Fig. 7.(a) that in upstream, mean ground water depth varied in the range of 10-13 m from mean sea level (MSL).

The ground water depth from MSL is seems to be inversely correlated with rainfall. As the rainfall amount increases the depth of water table from MSL has decreases. Fig 7.(b) indicated the similar kind of pattern of ground water behavious with respect to rainfall having avg. depth 6 to 7.8 m. The trend of ground water level wass also found increasing in middle region over the period of time.

The similar kind of pattern of ground water depth behaviour with respect to rainfall was found in downstream region. The avg. depth to ground water from mean sea level is around 5.5 m to 7 m in last 15 years

CONCLUSION

Insight of the current study, the spatial and temporal graphical analysis for the observation sites of the unconfined aquifer indicated that there is a good hydraulic connection of groundwater level with the rainfall. It can also be revealed that the rainfall has reasonable correlations with the groundwater level of Aquifer and suggesting a significant contribution of recharge from the rainfall sources to unconfined aquifer. The study also indicated that Bankeri block whichh lies in upstream followed by Babai block having maximium water fluctuation and depth in pre-monsoon & post monsoon which needs to be addressed in future for arresting groundwater declination further.

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Composition and quantity of cytotoxic waste from oncology wards: A survey of environmental characterization and source management of medical cytotoxic waste

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ABSTRACT

Interest in waste drugs as a part of hospital waste in relation to their negative impact on the environment has increased during the past years. Cytotoxic drugs play an important role in the treatment of various neoplastic conditions and are most often used in specialized departments such as oncology and radiotherapy units. In this study an initial inventory of pharmaceuticals and unused pharmaceuticals including hazardous waste drugs and antineoplastic (cytotoxic) chemotherapeutics was provided. By providing a questionnaire, the rate of cytotoxic consumption, residuals of drugs, vial and syringes, needles, gloves and the other cytotoxic waste was measured during a 30-day period in two oncology wards of Qom hospitals in Iran. The results determined that mean production rate of medical waste in two hospital is 435 kg/d and equal to 1.73kg/bed/d, including: 97% infection waste (1.67 kg/bed/d), 2.5% sharp and syringe waste (43.25 g/bed/d) and 0.5% pharmaceutical waste (8.65 g/bed/d). The rate of cytotoxic waste in the investigated hospitals was 293.5(gr/d) and equal to 0.07 total medical waste. On the other hand the average rate of cytotoxic waste in the oncology departments was 21.5 gr/bed and 16.5 gr / patient. The results determined that over 66% of residuals cytotoxic drug compounds can be converted in to nontoxic and no genotoxic by chemical degradation. Lack of awareness of health hazards, insufficient financial and human resources and poor control of waste disposal are the most common problems connected with healthcare wastes.

KEY WORDS: CYTOTOXIC-WASTE-QOM-COMPOSITION

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INTRODUCTION

Healthcare activities can lead to the generation of hazardous of wastes that may have adverse effects on human health and on the environment. Interest in waste drugs as a part of hospital waste in relation to their negative impact on the environment has increased during the past years. Many of the chemicals and pharmaceuticals used in health care are hazardous. They are commonly present in small quantities in health-care waste, whereas larger quantities may be found when unwanted or outdated chemicals and pharmaceuticals are sent for disposal, (Drug, 2015).

Considering the classification of hazardous healthcare waste by WHO the Categories of hazardous healthcare waste are: sharps, Infectious, Pathological, Pharmaceutical and cytotoxic, Chemical waste, and Radioactive waste. The types and nature of hospital waste depends upon the nature of the hospital and the service available in hospital (WHO, 2014). Exposure to genotoxic substances in health care may also occur during the preparation of, or treatment with, particular drugs or chemicals. The main pathways of exposure are inhalation of dust or aerosols, absorption through the skin, ingestion of food accidentally contaminated with cytotoxic drugs, ingestion as a result of bad practice, such as mouth pipetting, or from waste items. Exposure may also occur through contact with body fluids and secretions of patients undergoing chemotherapy. Genotoxic waste is highly hazardous and may have mutagenic, teratogenic, or carcinogenic properties. Genotoxic waste may include certain cytostatic drugs, vomit, urine, or faeces from patients treated with cytostatic drugs, chemicals, and radioactive material (Ansell, 2015).

Cytotoxic drugs (CDs) are primarily used as anti- cancer drugs because they are toxic to cells. These drugs have been associated with human cancers at high (therapeutic) levels of exposure and are carcinogens and teratogens in many animal species. They are most often used in specialized departments such as oncology units, whose main role is cancer treatment. Cytostatic drugs can be categorized as: alkylating agents which cause the alkylation of DNA nucleotides, leading to the cross-linking and miscoding of the genetic stock; antimetabolites which inhibit the biosynthesis of nucleic acids in the cell; and mitotic inhibitors which prevent cell replication (Antell, 2013). Medical waste is incorrectly managed throughout the majority of hospitals in Iran. Healthcare workers are not trained to conceive that a large proportion of medical waste generated in hospitals is Non-infectious waste. A structured waste management strategy together with clear definitions and staff training will lead to a decrease in waste volumes, and consequently to a reduction of costs in healthcare settings. Generated amounts of the health care waste are not available (for example Ireland) or the defined amount of wastes is too low (for example Bulgaria, Finland) or too high (in case of Belgium). Among other issues, this might be an indication of improper waste management practice or poor data collection in the country; Askarian et al, 2010; Farzadkia et al, 2009; Abduli, 2010 Nabizadeh, 2016).

In recent years, the rate of cancer disease and consumption of cytotoxic drugs in oncology wards of Qom hospital has increased. In this work composition, the quantity and possibility of chemical degradation of cytotoxic drug waste have been studied.

MATERIALS AND METHODS

This study was performed in 2015, in the oncology wards of two hospitals in Qom, Iran including Shahid Beheshti Hospital with 400 active beds and Hazrat Masoumeh Hospital with 120 active beds, located in the central part of Qom Province. The studied hospitals provide general medical, surgical, maternity, pediatric, and a range of specialist services.

Considering the methods of medical waste management and the main generation of medical waste in the two investigated hospitals, the total medical waste was classified in three categories: infection, sharp and Pharmaceutical waste. Several methods were used to collect data, namely site visits, interviews, and questionnaires. An initial inventory of pharmaceuticals and unused pharmaceuticals including hazardous waste drugs and antineoplastic (cytotoxic) chemotherapeutics was provided. Moreover, data collection consisting of healthcare waste generation, separation, collection, storage, transportation, and disposal was performed during site visits to the hospitals. With the cooperation and coordination of the personal and management of hospitals and using the questionnaire, the rate of cytotoxic consumption on, residuals of drugs, vial and syringes, needles, gloves and the other cytotoxic waste was measured in a 30 day period.

The Chemical degradability of cytotoxic waste was assessed with exposure to chemical oxidants (WHO, 2014). Specific physico-chemical properties such as: dissociation constant (pKa), solubility, octanol–water partition coefficient (Kow) and organic carbon partition coefficient (Koc), bio-concentration factor (BCF), atmospheric hydroxyl radical reaction rate and photolysis tendency play critical roles in determining the environmental behaviors and fate of cytotoxic waste (Cheng et al, 2009; Andrew et al, 2008; Toolaram et al, 2014; Zhang et al, 2013; Besse et al, 2012). The Prediction of environmental fate and other physico-chemical properties was carried by a theoretical model (EPI Suite 4.1)

(EPA, 2014). Two criteria contain octanol–water partition coefficient (Kow) and solubility was considered. A logKow<1suggests that the compounds are highly mobile in an aquatic environment. About solubility, adding or existance of polar functional groups that increase of hyrophilicity of drug compound were considered (Zhang et al, 2013; Besse et al, 2012).

RESULTS AND DISCUSSION

QUANTITIES OF MEDICAL WASTE GENERATION

There were 120 active beds in the Hazrat Masoumeh Hospital and the rate of medical waste was identified as 220 kg/d. Also there were 400 active beds in Shahid Beheshti Hospital with the medic production rate of 650 kg/d. Table 1 shows the average daily production of total medical in two hospitals. Medical waste from Hazrat Masoumeh hospital equaled 1.83 kg/occupied bed/d, of which 96.36% was infectious, 3.1% sharp waste and 0.45% Pharmaceutical waste .Shahid Beheshti hospital medical waste was 1.62 kg/occupied bed/d of which 98% was infectious, 1.7% sharp and 0.3% pharmaceutical waste.

Category of cytotoxic waste

The rate of cytotoxic waste was assessed in oncology wards of two hospitals separately. The results are shown in table 2.

Table 3 shows the mean production rate and category of cytotoxic drug waste in the hospitals under study.

Characteristics and chemical degradation of cytotoxic drugs

Twelve chemical structures of conventional cytotoxic drug compounds used in the oncology wards of hospitals under study were assessed. The results pertaining to the solubility and degradability of cytotoxic drug when exposed to chemical oxidants, the results are shown in Table 4.

Results of the present study determined that mean production rate of medical waste in the two hospitals was 435 kg/d and equal to 1.73kg/bed/d, including: 97% infection waste (1.67 kg/bed/d), 2.5% sharp and syringe waste (43.25 g/bed/d) and 0.5% pharmaceutical waste (8.65 g/bed/d). The results of study about hospital waste management status in Iran by farzadkia and at al showed that the waste generation rate was 2.5 to 3.01 kg bed(-1) day(-1), which included 85 to 90% of domestic waste and 10 to 15% of infectious waste. Waste generation rate in the hospitals varied from 1.25 to 14.8 kg/bed/d (Zhang et al, 2013; Besse et al, 2012).

Medical waste production depends on factors such as type of hospital, number of beds, socio-economic and cultural status of patients and waste management processes (Cheng et al, 2009). In Thailand, Italy, USA, India, Peru, Vietnam, and Tanzania 1, 3–5, 5–7, 0.5–2, 0.76–2.6, 1.42, and 0.84 kg/bed/d, of medical waste are respectively generated, are generated (Dehghani, 2008). According to a study of the composition and production rate of pharmaceutical and chemical waste in Greece, the production rate for total pharmaceutical waste was 7.48 g/bed/d (Voudrias, 2012).

Results of Table 2 and 3 exhibited that the rate of cytotoxic waste in the investigated hospitals was 293.5(g/d) equal to 0.07 total medical waste. On the other hand the average rate of cytotoxic waste on the in the oncology departments was 21.5 g/bed equal to 16.5 g/patient. Moreover, the total amount of generation waste from cytotoxic drug residuals was 120.2 mg/d (mean 4.92mg/d and standard deviation ±8.88mg/d for any cytotoxic drug) and the total amount other

Table 1. Estimated medical waste generation rate of hospital waste for two hospitals investigated

Name of hospitals	Number of active beds	Rate of total medical waste (kg/d)	Separation of medical waste		
			Infection waste (kg/d)	Sharp waste (kg/d)	Pharmaceutical waste(kg/d)
Hazratmasoumeh	120	220	212	7	1
Shahidbeheshti	400	650	637	11	2

Table 2. Rate of cytotoxic waste in the hospitals investigated

Name of hospitals	number of active beds in oncology department	rate of total cytotoxic waste	rate of cytotoxic waste	rate of cytotoxic waste
		(gr/d)	(gr/bed/d)	(gr/patient/d)
Hazrat masoumh	12	337	22.5	18
Shahid beheshti	15	250	20	15

Table 3. Average production rate and category of cytotoxic drug waste in hospitals investigate

Number	Drug compound	Daily average dosage used (mg/d)	Waste from residual of cytotoxic drug (mg/d)	Rate of other cytotoxic waste that produced from expose to cytotoxic drug (gr/d)
1	Cytarabine	120	8	25
2	Etoposide	100	6	18
3	Vincristine	170	8	24
4	Carboplatin	70	9	28
5	oxaliplatin	27	2.7	6
6	Fluorouracil (5-FU)	15	1.5	12
7	Cisplatin	120	3	8
8	Cyclophosphamide	170	18	16
9	bleomycin	60	7	20
10	Erinotekan	130	13	26
11	mesna	200	15	30
12	Dacarbazine (DTIC)	50	8	10
13	Methotrexate	130	10	25
14	L-asparaginase	80	8	10
15	Ifosfamide (Ifex)	170	17	25
Sum		1512	120.2	282

cytotoxic waste generated from contact was 282 g/d (mean 7.92g/d and standard deviation ±18.8g/d for any cytotoxic drug). Figure1 indicated that maximum production rate of daily average administrated dosage of cytotoxic drugs is related to Mesna (200 mg/d) and maximum production rate for waste from residuals is related to Cyclophosphamide (18mg/d)

Figure 2 with R^2 = 0.59 and Figure 3 with R^2 = 0.47exhibited a small correlation between daily average dosage and waste from residuals of cytotoxic drug and a smaller one daily average dosage and rate of other cytotoxic waste generated from contact.

The results of the by Tasakona showed that the rate of cytotoxic waste was 120 l/day and 0.03 from total

Table 4. Characterization of solubility and degradability of conventional cytotoxic drug compounds in oncology departments

Cytotoxic drug compound	Chemical Formula	Solubility of cytotoxic drugs with consider to polar functional group	Hydrophilicity of cytotoxic drug with consider to(Kow)
Carboplatin	$C_{15}H_{18}N_4O_3$	+	+
Vincristine	$C_{46}H_{56}N_4O_1$	+	+
Cyclophosphamide	$C_7H_{15}Cl_2N_2O_2P$	+	-
Cisplatin	$H_6Cl_2N_2Pt$	+	+
Bleomycin	$C_{55}H_{84}N_{17}O_{21}S_3$	+	_
Etoposide	$C_{29}H_{32}O_{13}$	+	+
Cytarabine	$C_9H_{13}N_3O_5$	+	+
Erinotekan	$C_{33}H_{38}N_4O_6$	+	+
Mesna	$C_2H_5NaO_3S_2$	_	_
Dacarbazine (DTIC)	$C_6H_{10}N_6O$	_	+
Fluorouracil (5-FU)	$C_4H_3FN_2O_2$	_	+
Methotrexate	$C_{20}H_{22}N_8O_5$	+	+

Notes: + sign exhibit that cytotoxic drug with log K_{ow} <1 or cytotoxic drug has polar functional group

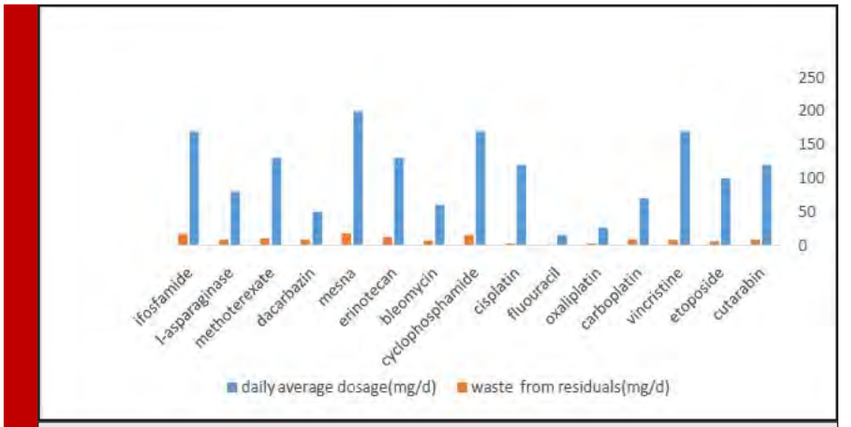

FIGURE 1. Comparison between daily average dosage and waste from residuals of cytotoxic drug waste

amount of medical waste, was different (medical waste density in Iran is 170 kg/m^3) but approve the Bill Brewer, Andrew Antel study that rate of cytotoxic waste from chemutraputic drug such as Cyclophosphamide (CP), Mitomycine, Mycophenolate was 0.08 ib/bed/day (Tsakona, 2010). Results of a survey by Evanglos Vondarials, suggested that rate of pharmaceutical's waste was 3.9% of hazardous waste in agreement with our results (Voudrias, 2012).

Cytotoxic waste residue as a source of cytotoxic drugs in the Environmental

With survey of medical waste management in two hospitals, direct disposal as the municipal solid waste is still a common way for the unused pharmaceuticals. Four primary sources (hospital effluent, household wastewater, and production discharge and drug waste disposal) of cytostatic residues. Hospitals produce large quantities of chemically- and which carry high potential ecotoxicity, and should not be considered as possessing the same pollutant nature as urban wastewater. The measured cytostatic levels in hospital sewage indeed correlated with the daily consumption and the pharmacokinetic excretion pattern (Zhang et al, 2013; Besse et al, 2012). At present study, results of prediction model (EPI Suite 4.1) was shown that, cytotoxic drug waste residue including: Carboplatin, Vincristine, Cyclophosphamide, Cisplatin, Bleomycine, Etoposide, Cytarabin, Erinotekan and Methoterexate with considering excration pattern and discharge in to hospital sewage, have been increased toxicity of aquatic environment (EPA, 2013).

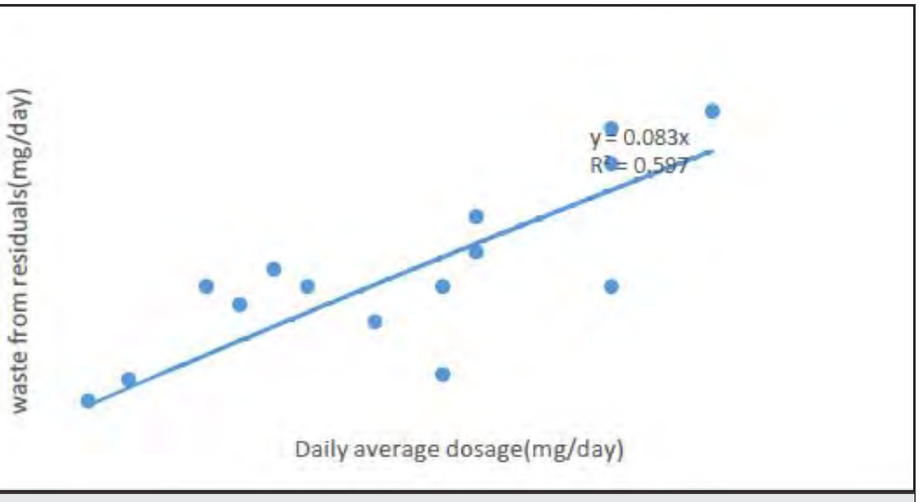

FIGURE 2. Daily average dosage versus waste from residuals of cytotoxic drug

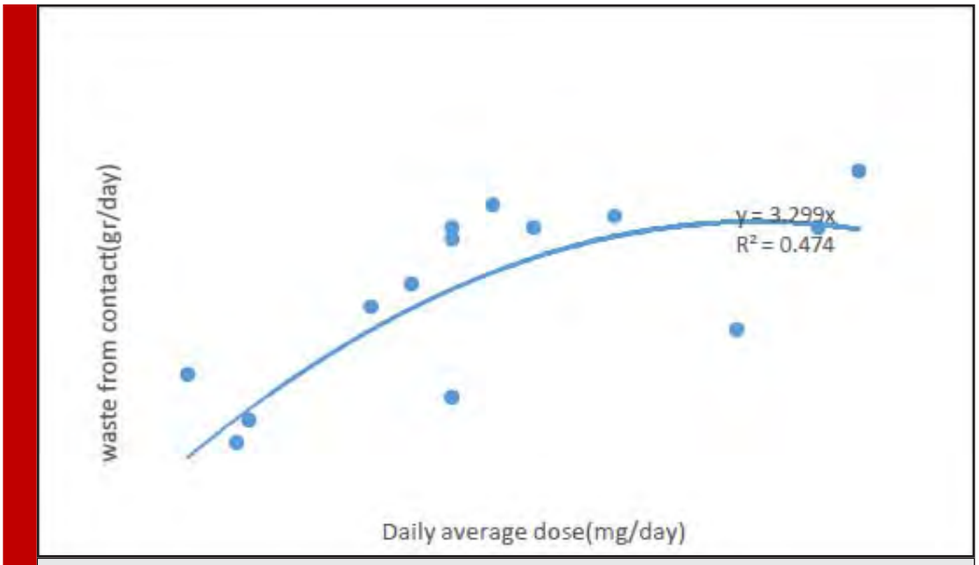

FIGURE 3. Daily average dosage versus rate of other cytotoxic waste from contact

Implementation of cytotoxic waste management plan and selection of treatment methods

Risk control measures for cytotoxic waste management including: identification, containment and segregation. Other requirements contain handling or storage of cytotoxic waste, disposal and treatment, in the process of waste management plan must be considered.

The choice of treatment system involves consideration of waste characteristics, technology capabilities and requirements, environmental and safety factors, and costs, many of which depend on local conditions. The results of study about_solubility and degradability of conventional cytotoxic waste is depicted in Table 4. For decrease of environmental risks and degradation of these compounds by chemical degradation methods as an option that appropriate for developing countries were investigated. results determined that over 66% of residuals cytotoxic drug compounds can be converted in to nontoxic and no genotoxic by chemical degradation including oxidation by potassium permanganate ($KMNO_4$) or sodium hypochlorite (NaCLO,5.25%) readily available in Iran hospitals. It must be noted that this process is not suitable for other cytotoxic waste containing vial, syringe, and gloves for which the appropriate process is handling (Drug, 2015).

CONCLUSION

The implementation of medical waste management is one of the most significant healthcare issues currently requiring attention in Iran. Hospital waste materials pose a wide variety of health and safety hazards for patients and healthcare workers. Many of hospitals in Iran have neither a satisfactory cytotoxic waste disposal system nor a waste management and disposal policy. Provision of a cytotoxic waste management planning and monitoring systems in hospitals is a prerequisite issue for effective reduction of health care waste associated risks.

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Fe3+-EDTA-zinc oxide nano-diagnostics: Synthesis and in vitro cellular evaluation

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ABSTRACT

Resulting from many efforts in opportune recognizing and correct treating briefly molecular imaging and therapy, some methods or molecules have been developed by now to overcome any unwanted defaults in imaging and therapy, specifically nanoparticles. Generally, synthesis new compounds, for example Zinc Oxide-Iron nano-complex consisting non-toxic paramagnetic ion [Fe3+] and its cellular uptake vehicle [zinc oxide] as a lowering risk of toxicity and increase in cellular uptake liability, could be useful and noticeable in molecular imaging purposes. The isolated nano-contrast was structurally analyzed by variety of techniques such as EDAX, AFM, Zeta and size measuring, SEM, FTIR and UV spectrums. Finally for monitoring the nano-complex toxicity and cellular uptake on human embryonic kidney cells named as HEK 293 was assessed respectively. The analytical result showed a very good promising size at nanoscale and zeta potential as well as iron content for the suggested ferric containing nano-complex as well as paramagnetic properties. Also outcome of cell study were resulted in no significant cellular toxicity comparing to 62% toxicity of control drug Magnevist and higher cellular uptake of 56% comparing to 9% for that of Magnevist as well. In summary, it seems that the proposed ZnO-Fe nano-complex may be useful as a novel low risk contrast agent to increase resolution in molecular Imaging like MRI and improve the current situation with the minimum cost.

KEY WORDS: SYNTHESIS, CONTRAST AGENT, MOLECULAR IMAGING, ZNO-FE3+ NANOCOMPLEX, HUMAN EMBRYONIC KIDNEY CELL

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INTRODUCTION

As per numerous endeavors in perfect perceiving and right treating [early conclusion and treatment], expansive quantities of creating strategies have been utilized as of recently. One of the imperative one in this field is sub-atomic imaging, an advanced innovation which gives legitimate component to the early recognition and portrayal of the ailments, checking of natural process in body, assessment of treatment and observing reaction, almost assessing drug pharmacokinetics. Atomic imaging as shows is the molecularly focused on, constant, and noninvasive imaging of wonders and procedures at cell and subcellular levels, (Meade et al. 2009). For upgraded determination execution imaging specialist as little particles, built protein nanoparticles has been performed, (James et al. 2012 Rameshwar et al. 2015 Langer et al. 2015).

At present there are a few imaging modalities usually utilized for atomic imaging like Magnetic Resonance which benefits balance operators with paramagnetic owing to properties and the others incorporate positron emission tomography (PET), single-photon emission computed tomography (SPECT), computerized tomography (CT), optical imaging [fluorescence and bioluminescence], photo acoustic imaging. Contrasted with other imaging modalities, the principle points of interest of attractive reverberation imaging is its great determination which can enhance by upgrading contrast specialist. The first kind of clinically applied radio-opaque usually known as contrast agents were salts of chemically designed complexes by paramagnetic diagnostics, such as Ferric (Fe III), gadolinium (III) and manganese (II) which their mechanisms show that such complexes of mentionedparamagnetic radio-metalsdecrease the longitudinal (T1) and transverse (T2) relaxation parameters of surrounded water molecules, (Lauffer et al.1987), (Toth et al. 2002) (Schwert et al. 2002).

Indeed it is notifying about paramagnetic compounds as chemically designed agents ready to go about as differentiation operators in the locales where they disperse in body and improve differentiate amid imaging. Among the difference operator the greater part of them are low sub-atomic weight metal edifices. Also the utilization of paramagnetic metal for this propose, nanomedicine formulation by employing different types of nanoparticles such as polymeric or metal based such as zinc oxide nanoparticles have been vastly used for imaging applications beside for therapeutic goals. Little size of nanoparticles encourage the auspicious identification of little different changes furthermore give a high surface range to stacking different atoms. Attractive nanoparticles have additionally been utilized to convey medications to a sick range, (Barakat, 2009).

It is another test utilizing nanoparticle as a part of both indicative and restorative objectives. In such framework the name as theranostic has been more recognizable in scientist's mentality, nanoparticles assume an essential part as growing high flag force and limit with regards to various application, (Jain et al. 2008). The fundamental advantages of utilizing nanoparticle as a part of symptomatic and imaging are low lethality, site-particular collection and hours of course time next to their wellbeing and biocompatibility, (Jesse et al. 2011). In spite of broadly utilization of difference operator up to now, many looks into are progressing due symptoms and lethality of complexity specialist especially in crucial organs, for example, kidney and here and there low determination that are the primary issues as dependably uncommonly in the individuals who utilize this strategy to catch up the treatment procedure forever. Even so, synthesis new compounds with all the ideal index have still been restricted and have no complete in vitro or in vivo data. Herein, we synthesis a new Compound including Zink Oxide nanoparticle in chelation with Fe^{3+}-EDTA in optimal condition with the aim of creating low risk contrast agent and improving the current situation along with minimum cost. Briefly in current experimental observations, stable complex of iron with a chelator was formed and then such complex loaded into zinc oxide nanoparticle for increase in cellular uptake liability of tumors.

MATERIAL AND METHODS

Zincsulfate hepta hydrate [$ZnSO_4.7H_2O$, Merck], Sodium Hydroxide [Merck], Deionized Water, EthyleneDiamin Tetra Acetic acid [99.5 %, Merck], Ferric-Cholorid hexa hydrate [$FeCl_2 .6H_2O$, Merck] were used for the synthesis of the materials. In the following, scanning electron microscope [SEM] with EDAX analysis was provided by Phenom-Prox model made in Holland Atomic-force microscopy[AFM] image was obtained by CP-RESEARCH[CP-R] model, VEECO Manufacturer Company made in America. Zeta potential and size on nanoparticle in water as a solvent and ultimate nanocomplex in DMSO as a solvent were performed by Malvern Nano-Zetasizer from Pasteur Institute in Tehran. Fourier Transform Infrared spectroscopy [FTIR] result was measured by Perkin Elmer, Spectrum Two FT-IR and at the end ultraviolet-visible spectrum was measured on VARIAN, CARY 100 Bio UV-VIS. Cells and related mediums were provided from Pasteur Institute of Iran.

SYNTHESIS OF ZINC OXIDE NANOPARTICLE

In order to synthesize ZnO nanoparticle water based solution [0.2 M] of zinc nitrate as well as a prepared

[0.4 M] of NaOH was prepared by using deionized water, respectively. The accessible NaOH solution was dropped into zinc nitrate medium at room temperature[rt] with accurate stirring, which advanced in the accumulation of abeyance with a white color. Resulting white finished compound was elaborately centrifuged at 5000 rpm for at least 20 min and then washed three times with eluting solution of distilled water as well as washing with absolute alcohol finally. The acquired artefact was finally calcified at 500 °C in air atmosphere for 3 hrs to achieve zinc oxide NP respectively.

SYNTHESIS FE^{3+}-EDTA COMPLEX

1 mol EDTA equivalent 0.25g [M=292.2 g/mol] was mixed with 20ml distilled water and after 5 minutes of stirring, 1 mol Ferric Choloride hexa hydrate equivalent 0.27g [M=270 g/mol] was added to the container. The mixture was heated to 90 ^{0}C for 10 minutes with stirring. Then after wrapping the foil placed 7-10 days without moving in laboratory condition to form the intended complex. Eventually purified by sephadex G25 column or Dialysis bags [cut off 1000Da] as well.

SYNTHESIS ZNO-FE-EDTA NANOCOMPLEX

To synthesis the ultimate complex, half of the amount of Fe-EDTA complex was added to the double amount of Zinc Oxide nanoparticle with 5-7ml distilled water and were stirred for 20 minutes to obtaine light brick color transparent solution and placed 7-10 days without moving in 25^{0}C, covering with aluminum foil. Henceforth purification by sephadex G25 was performed as previous step.

In order to confirm the final structure, nanocomplex was structurally analyzed by variety of techniques. To characterize size, morphology, elements Scanning Electron Microscopy[SEM] withEDAX analysis was used, andcalculation of the roughness parameters measured by means of the Atomic Force Microscopy[AFM], Zeta and size measuring ,also Fourier transform infrared spectroscopy[FTIR] is an established tool for the structural characterization of the new complex and in additionUltraviolet[UV] spectrum was used to identify the active site in nanocomplex, measuring zeta potential and size of nanoparticle was done eventually.

IN VITRO CELLULAR TOXICITY [XTT]

In order to find and assesse the possible toxic parameters of synthesized zinc oxide nanoparticles containing trivalent iron-EDTA, XTT method was employed as described in detail in the literature. Furthermore, as stated in literature one of the possible route of discretion of nanoparticle from the body as well as major affected body part of human by the contrast agents is kidney, HEK-293 was elected for XTT experimental analysis and observations based on the effects of different dosages of zinc oxide nanoparticles containing trivalent iron-EDTA, Fe^{3+}-EDTA and Magnevist [as a FDA approved MR contrast agent] as controls. XTT data were performed for 72 h period of time.

IN VITRO CELLULAR UPTAKE

To evaluate the cancerous cellular permeability of zinc oxide nanoparticles containing trivalent iron-EDTA, MCF-7 was chosen and different dosages of zinc oxide nanoparticles containing trivalent iron-EDTA, Fe^{3+}-EDTA and Magnevist [as a FDA approved MR contrast agent] as controls were assessed at an obvious time of 6 hours after exposure. After a subjected times cells treated by the agents were eluted by cell culture medium and the cells were centrifuged and lysed by acidic medium. The resultant solution was transferred to detect amunts of Gd^{3+} and Fe^{3+} by ICP-Mass spectroscopy as well. Intercellular concentration of metals were considered as percentage of cellular uptake.

RESULTS AND DISCUSSION

In Figure 1 SEM with EDAX analysis for ZnO nanoparticle is presented, in addition the image of the sample in various dimension, the result of SEM also be displayed as spectrums. The EDAX pattern exhibited different peaks that each of them shows a specific element percentage. The higher peak represents greater concentration. In this way, each element has a special atomic structure containing series of peaks. We have followed the same procedure after preparation of Fe-EDTA complex and ultimate nanocomplex and prominent additional peaks for new elements are obvious whereas those peaks are completely absent in the case of EDAX spectra of the ZnO nanoparticle.

In Figure 2 AFM topography of the as prepared ZnO nanoparticle and the ultimate nanocomplex are presented. The diagram and two-dimensional images of the initial nanoparticle and conclusive nanocomplex are shown in Figure 2a-b which shows the approximate the height of the sample. Three-dimensional images of ZnO nanoparticle and final complex are displayed in Figure 2c-d that shows peak-valley more concretely. The Fe-EDTA complex is inside ZnO nanoparticle pores randomly resulting in the formation and size of nanocomplex. As the load increases, the values of area roughness, line roughness, and also peak–valley height increases obviously. Thus surface morphology verification of nanoparticle and terminal nanocomplex was determined by AFM technique. The changes are explicated by

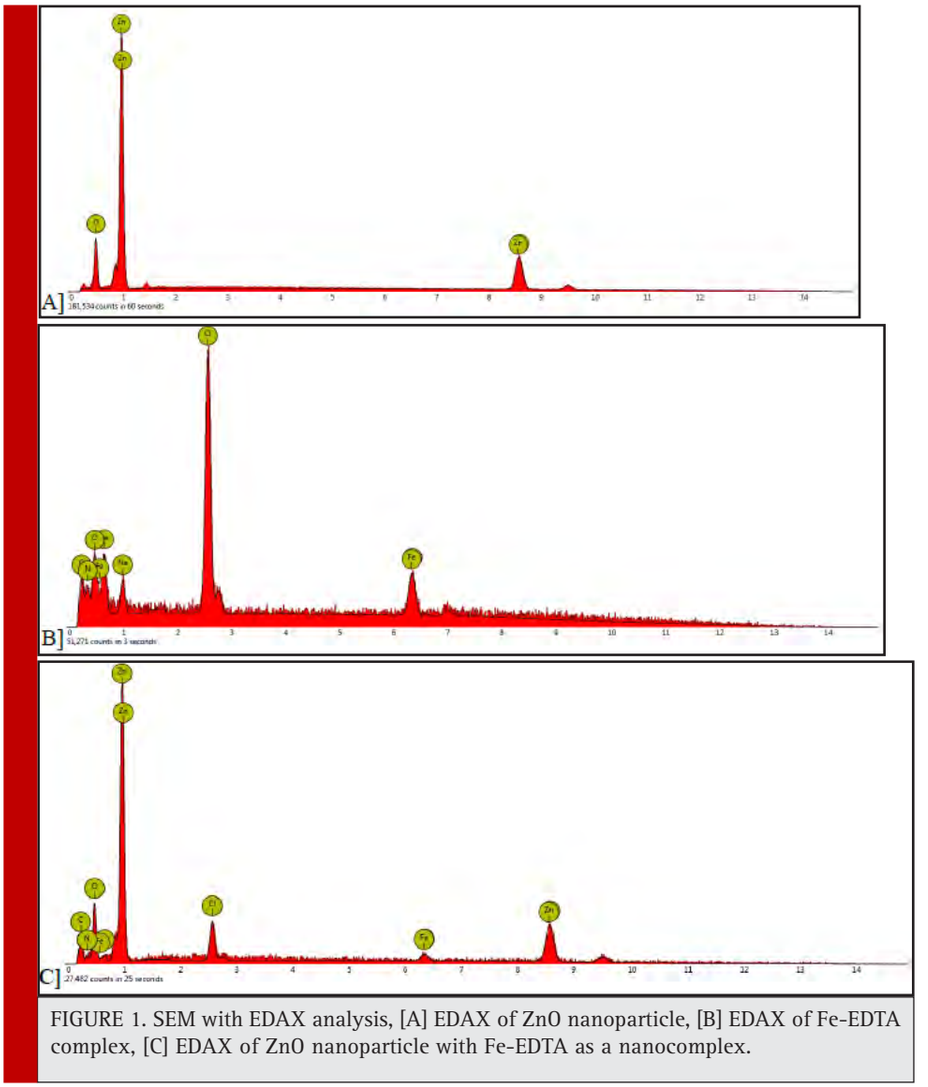

FIGURE 1. SEM with EDAX analysis, [A] EDAX of ZnO nanoparticle, [B] EDAX of Fe-EDTA complex, [C] EDAX of ZnO nanoparticle with Fe-EDTA as a nanocomplex.

comparing the graphs. Another physicochemical parameter which has been investigated by Zetasizer is particle size and zeta potential. The average diameter, particle size distribution index, intercept of ZnO nanoparticle and final nanocomplex is shown in Figure 3 and in addition the nanoparticles charge surface before and after reactionsare-7.31mv and -5.63mv respectively.

The FTIR spectrum support the result obtained from SEM, AFM, Zetasizer. After each reaction new peaks appear that proverecent atoms and links as an example a FTIR absorption band is observed at 3436 cm^{-1} which represents NH link related an amino group of EDTA or the peak in fingerprint zone, about 440 cm^{-1} reveals presence of halogen of $FeCl_3$ in final nanocomplex and background spectrum of ZnO nanoparticle including a peak at 2926 cm^{-1} confirms the presence of zinc oxide nanoparticle.Figure.4 give more explanation about spectrums.

At the end UV-VIS spectra absorption is recorded between 200-800nm wavelengths. In Figure 5 there is a clear indication of Fe-EDTA complex in the absorption spectrum after loading. The absorption spectrum of the Fe-EDTA complex shows two peaks with absorption between 1.5-2.5 which the first one is related to Carboxylic Acid group of EDTA and the second one demonstrates Ferric Choloride that because of its orbital properties is tended to long wavelengths or visible light. Also the final nanocomplex dissolved in DMSO shows a sharp and narrow peak between 0.2-1nm comparably the previous one.

Moreover, as stated in Figure. 6 zinc oxide nanoparticles containing trivalent iron-EDTA, Fe^{3+}-EDTA at all the

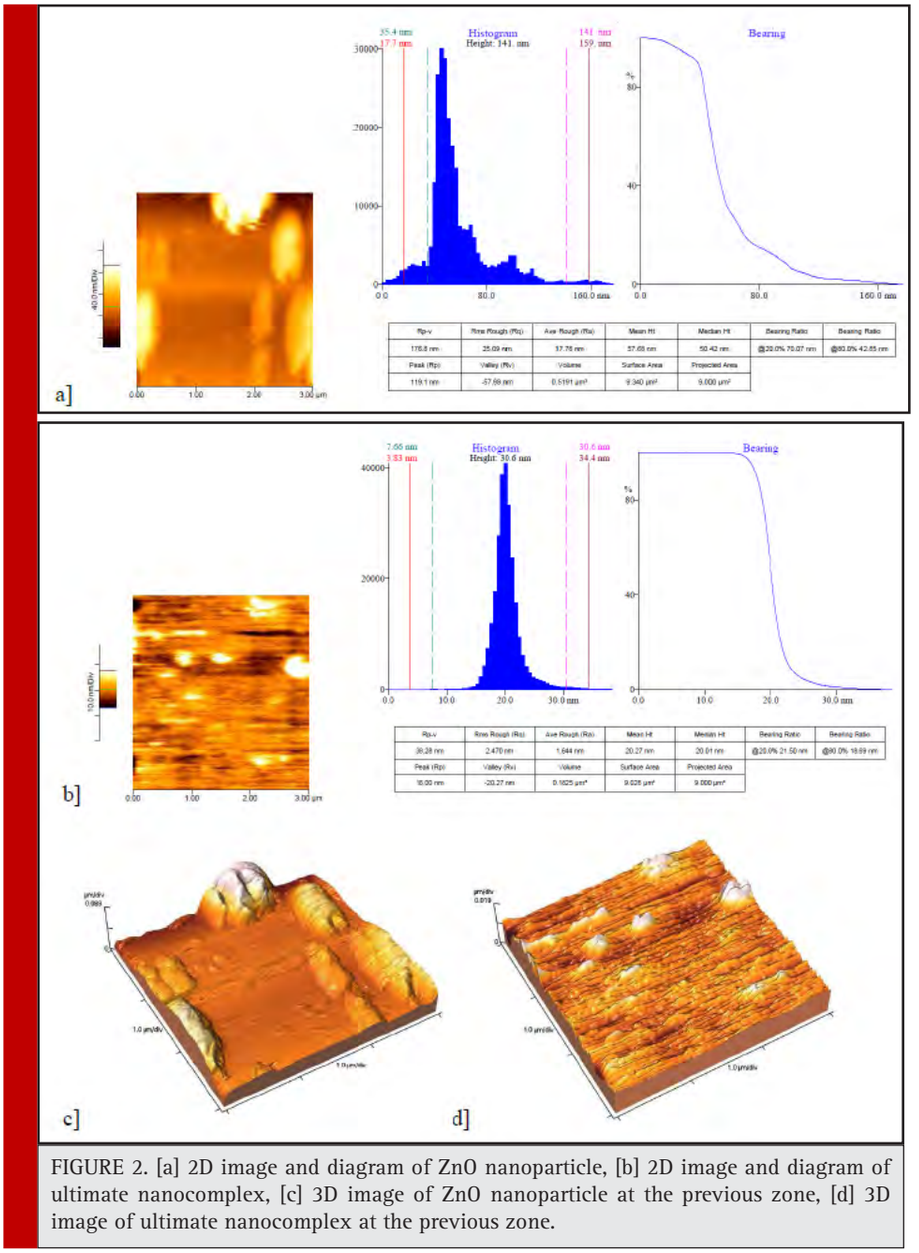

FIGURE 2. [a] 2D image and diagram of ZnO nanoparticle, [b] 2D image and diagram of ultimate nanocomplex, [c] 3D image of ZnO nanoparticle at the previous zone, [d] 3D image of ultimate nanocomplex at the previous zone.

used dosages did not rise any significant toxic effects on human kidney cells otherwise a significant toxic effects was shown by Magnevist 100 µM [as a FDA approved MR contrast agent] as control caused 62% cellular mortality respectively. Additionally a very good promising cancer cell uptake was recorded for zinc oxide nanoparticles containing trivalent iron-EDTA 56% comparing to 9% for that of Magnevist and 5% for Fe-EDTA as well [more details Figure 7].

As it is clear utilizing contrast specialist as a part of sub-atomic imaging is a discussable subject in atomic prescription, so profited a low dangerous balance operator with ideal impact has dependably been a worry. Too Many diverse diagnosis of some targeted human body sections in molecular imaging methodology including Magnetic Resonance Imaging MRI showed noticeable drawbacks that could be considered as difficulties to remain them also without other confirmation techniques that mostly would be invasive or even defined as impossible, (Rameshwar et al. 2015).

Nephrotoxicity and unfavorably susceptible response of many difference operator are the other test because

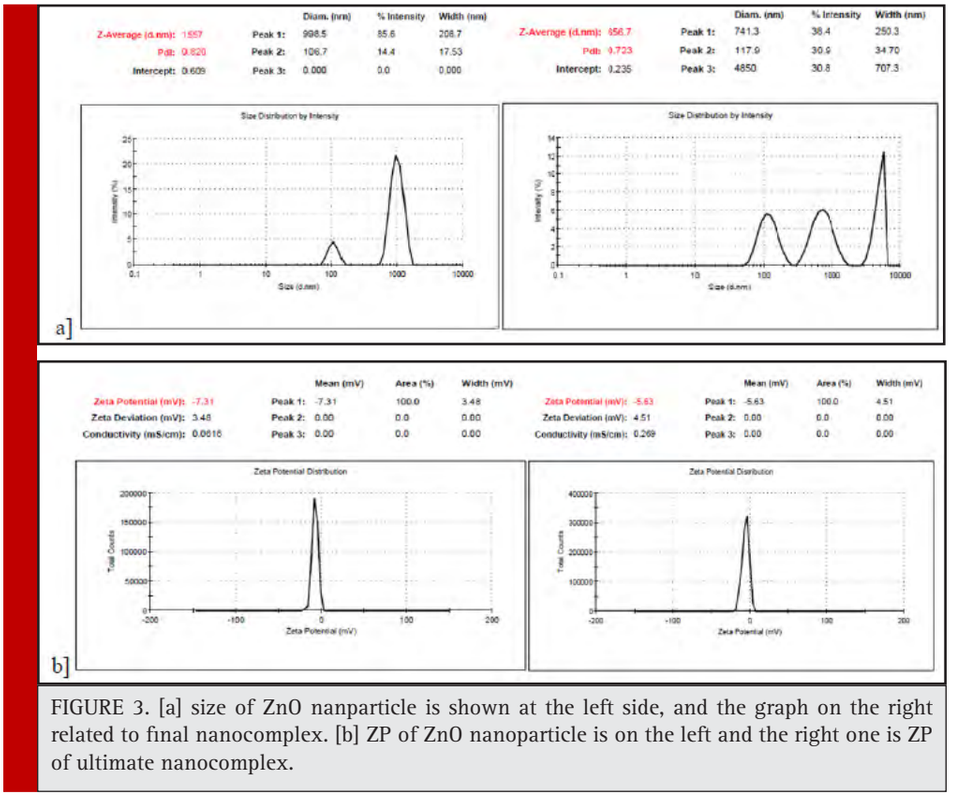

FIGURE 3. [a] size of ZnO nanparticle is shown at the left side, and the graph on the right related to final nanocomplex. [b] ZP of ZnO nanoparticle is on the left and the right one is ZP of ultimate nanocomplex.

of their structure and phisico-concoction portrayal. In such cases FDA caution is not immaterial showing the improving shot of Nephrogenic Systemic Fibosis [NSF] and or Nephrogenic Fibrosing Dermopathy [NFD] for the determined patients confounded to have renal disappointment maladies. [Enzo et al. 2010] What's more moderate discharge and high lethality of numerous macromolecular complex, for example, Gd III, are because of the digestion system which can discharge Gd III particle in body, (Zheng-Rong et al. 2003). Magnetic nano-bio-materials like fe-EDTA-prepared zinc oxide NP nanocomplex using in MRI have been well offered for the next subsequent imaging such viable tumor places (Langer et al. 2015) then again different techniques which require keeps imaging amid treatment. However nano-drugs like nano-complex can defeat natural obstructions which can impact on conclusion and treatment because of passing particles. Then again this nano scale give a fast dissemination amid systemic conveyance furthermore can be cleared up easily (Frank et al. 2008). These qualities gather least danger and time to get the most extreme impact and tissue aggregation. Numerous molecule in view of nanotechnology have been considered to conform the pharmacokinetic properties furthermore upgrade the relaxivity which metal-based nanoparticle can be noted, (Enzo et al. 2010, Na et al. 2008).

In this work we have used $FeCl_3$ chelating with EDTA, a six dentate ligand, and react the resulting complex to ZnO nanoparticle to be placed in its pores and structure properly. Ferric as main recognized paramagnetic ionable to provide adequate magnetic effects on surrounding water atoms and causes to reduce in T_2 and or T_1 relaxation time quantities. Subsequently this decline makes a brighter picture in light of T1 relaxation time lessening and darker picture consequently diminishing T2 relaxation time, (Brown et al. 2003) (Na et al. 2008). Contrasting with present difference operator, Gd buildings are delegated the specialists that impact on T1 relaxation time and can't wide water proton resonance and also T2 specialist, for example, paramagnetic and superparamagnetic specialist containing iron. [(Luca et al. 2012)) (Na et al. 2008)]. So creating new compound by having both T1 and T2 diminishment can be so useful. Therefor in this paper we synthesized ZnO-Fe nanocomplex besides EDTA as a safe FDA approved and low cost effective chelator in optimum condition.

The result of physico-chemical study showed a good and acceptable data and also low toxicity, at least cost and availability of the material needed are the other positive aspects. The small size of nanocomplex and existing Fe^{3+} as a paramagnetic agent in ultimate complex displayed a stable and appropriate complex in different

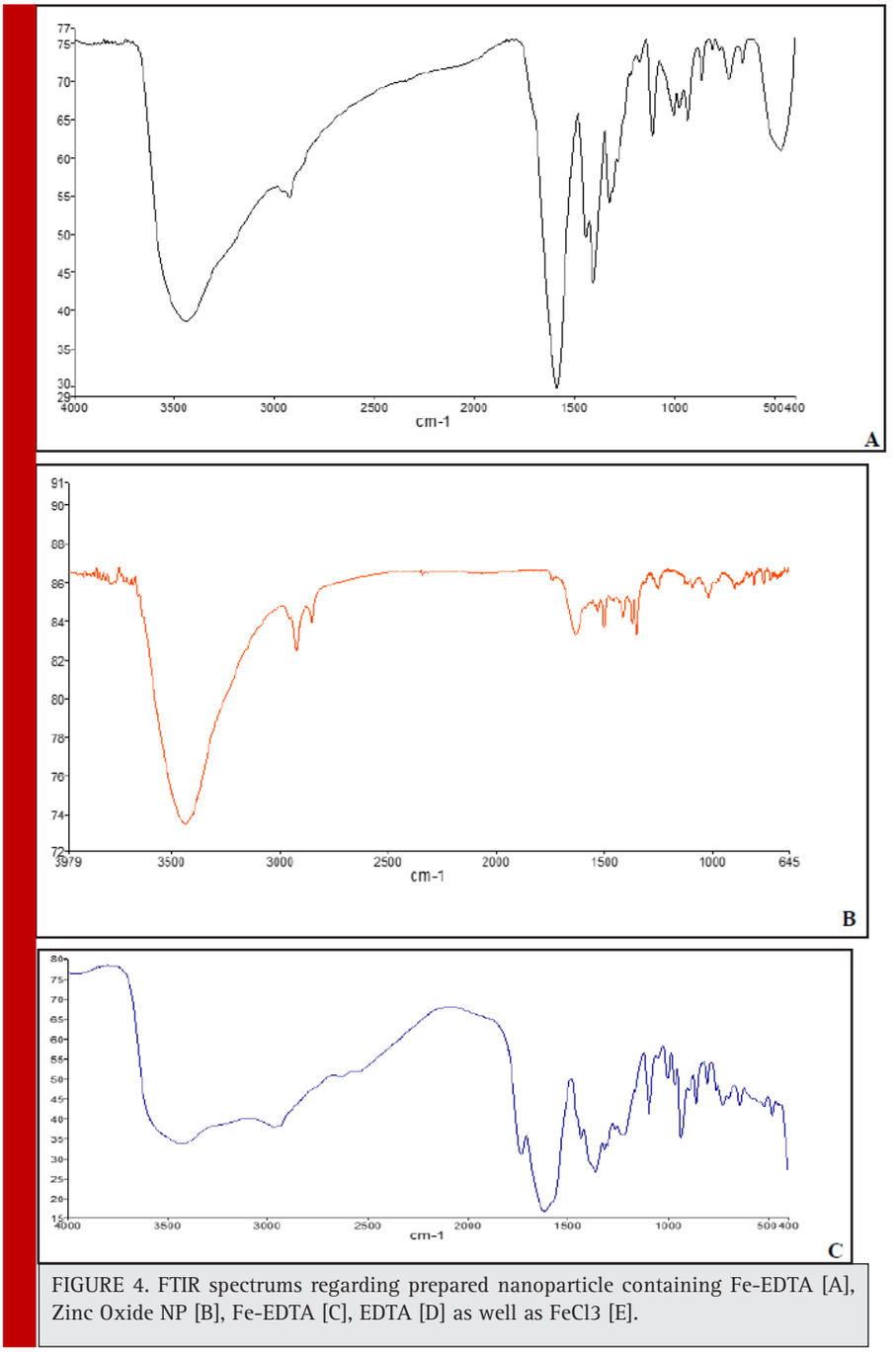

FIGURE 4. FTIR spectrums regarding prepared nanoparticle containing Fe-EDTA [A], Zinc Oxide NP [B], Fe-EDTA [C], EDTA [D] as well as FeCl3 [E].

features such as size, surface charge, topography, functional groups and fundamental elements. Ease of entry and low risk of cytotoxicity make this nanocomplex as a novel carrier in loading some drugs and having therapeutic effects in addition to diagnostic effects especially in tumor cells with prominent uptake.Consequently, based on these points and outcomes synthesized ZnO-Fe nanocomplex can freely play an important and effective role in the replacement of present contrast agent in MR molecular imaging erelong.

A novel nanocomplex of ZnO-Fe along with EDTA has been prepared by a simple chemical approach as a low toxicMR contrast agent. Thus after chelating iron by EDTA, composed complex reacts with ZnO nanoparti-

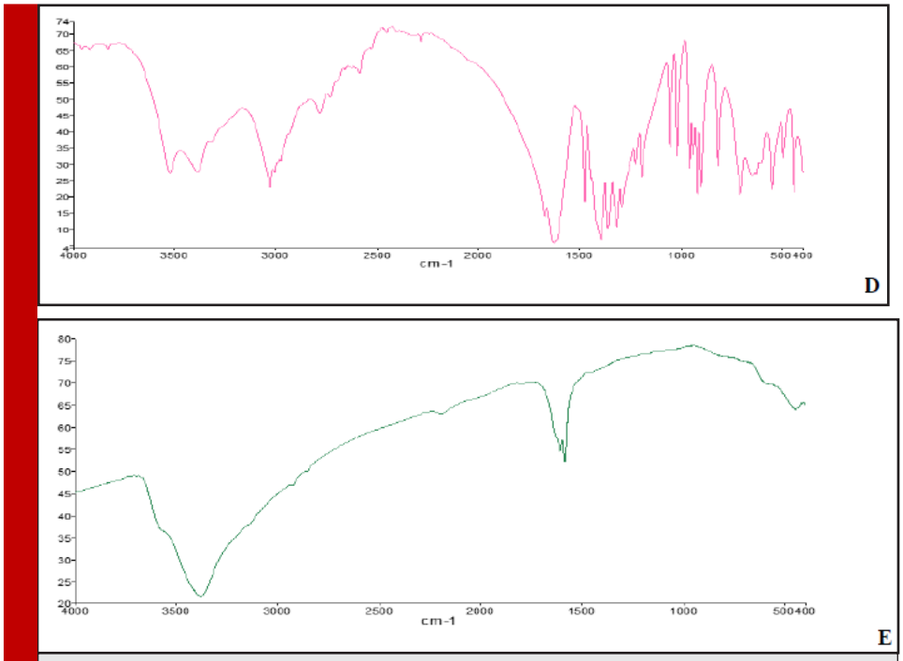

FIGURE 4. (*Continued*)

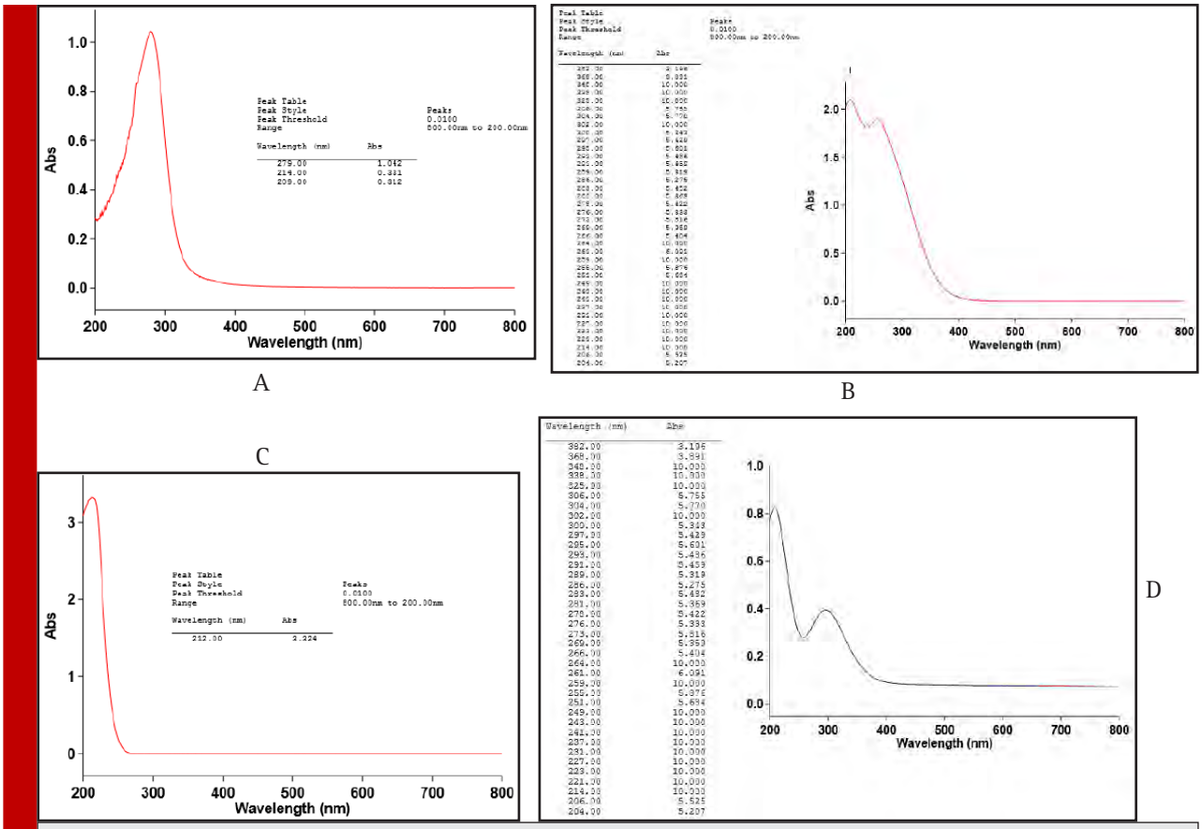

FIGURE 5. U.V spectroscopy data regarding prepared nanoparticle containing Fe-EDTA [A], Fe-EDTA [B], EDTA [C] as well as FeCl3 [D].

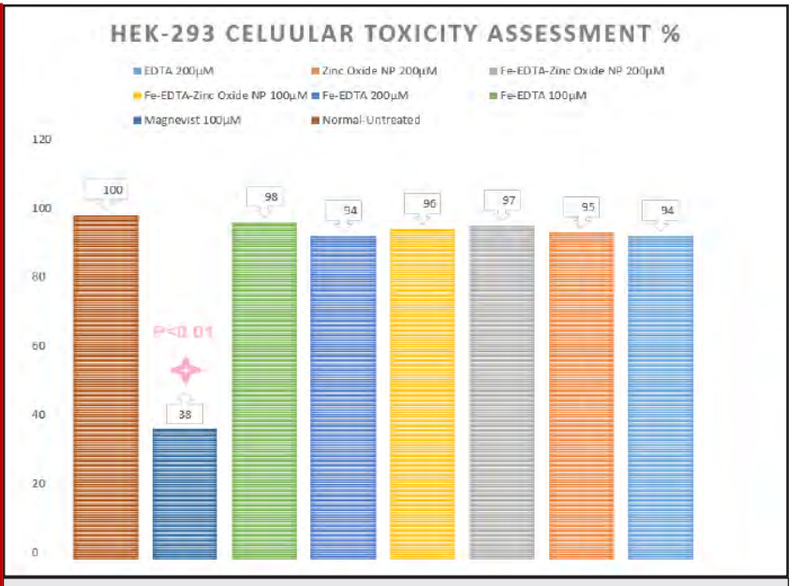

FIGURE 6. Illustrations of HEK-293 Cellular Toxicity resulting from 72 hrs. Exposure to different concentration of Magnevist, Fe-EDTA and Fe-EDTA-Zinc Oxide NP respectively. Potential significant toxicity regarding to Magnevist was observed otherwise no toxic effects were recorded for proposed NP as well.

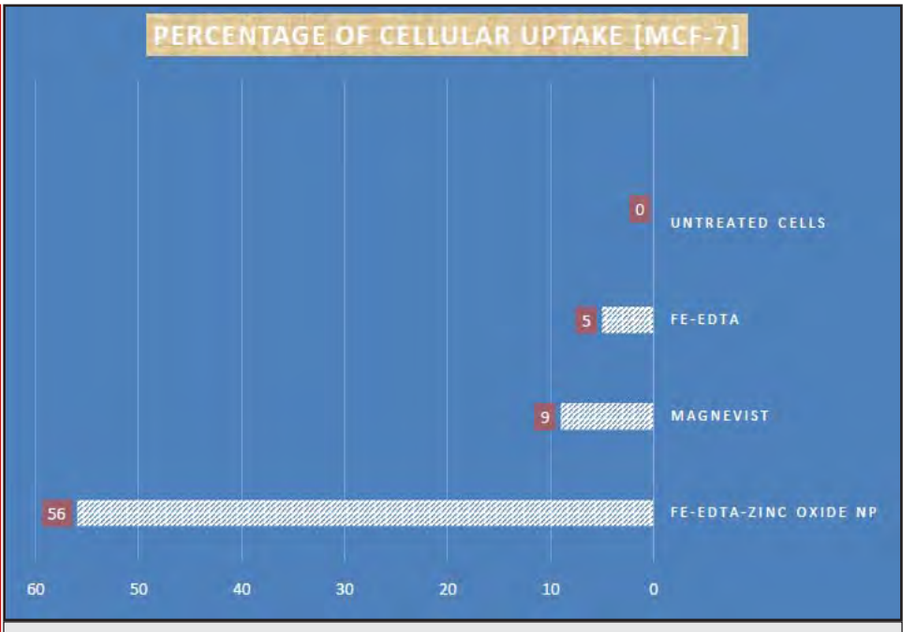

FIGURE 7. Depiction of competitive MCF-7 cellular uptake regarding Magnevist, Fe-EDTA and Fe-EDTA-Zinc Oxide NP respectively. Obtained data were recorded from a period of 6hr at the same concentration of 50 µM.

cle and a new stable nanocomplex is formed. Small size of ZnO in nano scale helped to uptake more by cells. The presence of Ferric Chlorideas a valuable magnetic agent makes opportune recognizing easier in molecular imaging like Magnetic Resonance Imaging. The physicochemical characteristics were monitored by different analysis resulting in desired achievements that confirm the final nanocomplex without any structural damage. In conclusion, along the development of nanoparticles for targeted diagnosis and therapy for biomedical application, proposed ZnO-Fe nanocomplex may be useful as a novel low risk contrast agent to increase resolution in molecular Imaging like MRI and improve the current situation with the minimum cost. Considering lower normal human cellular toxicity as well as higher and promising cancerous human cellular uptake comparing to Magnevist it could be concluded that such iron containing nanoparticle marked as new target for future in vivo studies as well as clinical practice.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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Comparison of stress distribution around implants with three different attachments in overdenture supported by four maxillary implants using finite element analysis method

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ABSTRACT

As a general rule, the purpose of treatment planning should be minimization and evenly distribution of mechanical stress in the adjacent implant and bone system. Various experimental studies have examined the distribution of stress in the implant supporting bones, thus avoiding the dangers involved. But there is still controversy about biomechanical effects and stress distribution in different attachment designs. The purpose of this study was to compare the distribution of stress around the implant with three different attachments in overdenture based on four maxillary implants using finite element analysis method (FEA). In this experimental study, using the FEA method, a 3D model of maxilla, implant, attachment components and overdenture was first prepared and then, using the ANSYS finite element software, the components of the model were superimposed on each other so that it can act as a component integrated with different materials. Implants are located in the anterior and posterior parts of the maxilla, with two implants placed in the two sides of the canine, one in the left central position and the other in the right second premolar. These implants were attached to the overdenture using three bar-clip, ball, and locator attachment designs. The 100N force used in this study and imposed vertically to the tooth 6 unilaterally and bilaterally and the stress level was investigated in each design. Among the three different designs examined, the highest stress was observed in the vertical force (22.87 MPa) imposed on the implant in the right second premolar on the right overdenture supported by the locator implant. In the second place, the highest stress was observed on the Ball-retained overdenture in the right side force, imposed on the implant in the right second premolar corresponding to 12.88 MPa. The least stress among these three designs was observed in the bar-clip-retained overdenture design, the maximum stress on which is in the vertical force imposed on the right side to the right second premolar implant at the size of 7.486MPa. Bar-clip-retained overdenture with the lowest stress caused by the 100 N force is the most appropriate design in the present study.

KEY WORDS: FINITE ELEMENT METHOD; STRESS TEST; BAR-CLIP ATTACHMENT; BALL ATTACHMENT; LOCATOR ATTACHMENT; OVERDENTURE

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INTRODUCTION

Considering the aging of people in communities and consequently the increase in complete edentulousness, today edentulousness has become a public health problem. Unfortunately, in Iran, edentulousness is not seen only in the elderly and many young people also suffer from complete edentulousness for a variety of reasons, including systemic diseases, non-compliance with oral and dental care, and absence of periodic visit to the dentist. Edentulousness can reduce the quality of life of patients both physically and psychologically. Recent studies have shown that tooth loss can affect the intake and absorption of nutrients due to reduced chewing ability, and increase the risk of multiple diseases. For this reason, dental implants are used to improve the chewing efficacy in complete edentulous patients and have improved the quality of life of these patients to an optimum level, (MacEntee et al 2003, Abnet et al., 2005, Semba et al 2006, Goiato et al 2008, Pennington et al 2012, Cunha et al 2013 Cakir et al 2014, Dezhdar et al 2017 and Geerts 2017).

The treatment that is predominantly performed for edentulous patients is the use of complete denture hands. From a public health perspective, although this treatment is a simple and inexpensive treatment, it cannot, be a general remedy for all patients. One of the main problems in edentulousness patients is their low satisfaction in denture use. The main reason for this discontent is poor compliance, inadequate collision, ulceration and pain (Zarb et al 2004). Considering the problems mentioned for ordinary complete dentures, a rational solution must be sought to the problems. Two types of treatment are widely used for these patients: there are fixed implant supported prostheses and implant-dependent overdenture that are widely used for complete edentulous patients (Dias et al 2013) and the use of any of these treatments depends on the patient's characteristics and conditions, including the amount of bone remaining, intervertebral space, oral hygiene, cost, and patient satisfaction (Zafiropoulos et al 2010).

Fixed implant-based prostheses may be one of the best ways to treat edentulousness and are implemented in case of sufficient bone and mandibular space (Chee et al 2006). More implants are usually needed to support a fixed prosthesis than an overdenture (Payne et al 2009). Since it is sometimes impossible to use a large number of implants for the patient, this issue limits the use of fixed prostheses (Chee et al 2006). Other constraints on the use of fixed prostheses is the loss of facial beauty due to the lack of lip support and soft tissue face, lack of access to hygiene, multiple and high cost surgical procedures (Vogel 2007). Using overdentures has greatly resolved the problems associated with the use of fixed prosthesis. Implant-based overdenture is a moving prosthesis that is placed on smaller number of implants per jaw, and has excellent attachment and stability. Overdenture is a simple, cost-effective, durable, less invasive treatment and a successful treatment option for edentulous patients and its use has recently become very common (Assunção et al 2008). Implant-based overdenture has improved the function of implant therapy (Awad et al 2003) due to the benefits of physical and natural beauty, and is superior to conventional dentures in many cases (Sadowsky 2001). Different studies have shown that this type of treatment has improved general health and quality of life of patients (Awad et al 2003). So, although the patient wants a fixed implant-based prosthesis, (s) he tends to use overdenture. Implant-based overdentures are connected to the implant by an interstitial part called attachment, which allows the prosthesis to resist against displacement forces (Locker 1998).

Many attachments such as rods, buttons, and magnets, are used to support implant-dependent overdentures (Machado et al 2011). Clinical success and longevity of dental implants are affected by the distribution of stress transferred to the implant and surrounding bones (Jacques et al 2009). Also, the lack of sufficient biocompatibility between the implant and the surrounding bone can lead to implant failure (Berglundh et al 2002). Different attachment systems used in overdentures show different biomechanical characteristics and can be dangerous to implant supporting bones (Sadowsky 2007). Various experimental studies have examined the distribution of stress in the implant supporting bones, thus avoiding the dangers involved. But there is still controversy about biomechanical effects of stress distribution in different attachment designs (CHUN et al 2005) and (Baumeister 1978). Paying attention to the principles of biomechanics in implant-based prosthesis can provide a suitable treatment design for each patient and reduce the probability of functional problems or implant failure (Baumeister 1978). Different methods are used to evaluate the stress and strain in the bone around the implant, which can be used for photoelastic analysis, strain gauge, and finite element analysis.

Fine Element Analysis (FEA) is a precise method for evaluating the amount and pattern of stress distribution in dental structures which has many advantages over other methods. Precise geometric reconstruction of the structures involved in stresses acceptance and distribution, ability to accept and receive different simply, showing the procedure of internal stresses and other mechanical quantities, rapid and simple repeatability of the tests are among the benefits of this study method. FEA is a numerical and quantitative method for analyzing stress in complex structures. In this method, separate structural elements are connected through points

or nodes. These components are created by dividing the primary structure into units of the proper shape. For each element, the physical properties are considered proportional to the simulated material (Powers 2006).

This method is also used in dental studies as an ideal method for preparing the correct tooth model and it's supporting structures in three dimensional form. This method can provide measurement of partial mechanical responses towards the difference in mechanical parameters and the evaluation of stress in dental materials and tissues at different levels (Motta et al 2006). Various studies, using the FEA method, have shown that stress distribution in maxillary and mandibular implant systems is significantly affected by various attachment designs (CHUN et al 2005). The maxillary implant supported-overdentures are less predictable than mandibular overdentures, and there is controversy over the use of different designs and the number of implants used (Dudley 2014). One of the overdenture treatments in maxilla is the use of four implants, which has been studied in few studies. There are also few studies on maxillary overdenture than the mandibular one (Raghoebar et al 2014).

Some studies have shown that 4 or more than 4 maxillary implant-dependent overdentures are more stable and more durable than less than 4 implant-dependent overdentures (Raghoebar et al 2014). These implants can hold the overdenture with various attachments. Results of studies on the acceptance and use of various attachments showed that the bar and button attachments that have a good stability as well as locators that are newer, are at a higher level in terms of general acceptance and enjoy more clinical use (Büttel et al 2009) and (Lončar 2015). For this reason, these attachments will be used in a four-implant design to measure stress in the present study. The present study uses a FEA method to simulate the structure of various overdenture designs based on four maxillary implants, to investigate the distribution of Von Mises stress in the surface of all attachments and implant supporting bones so that the most suitable solution for the treatment is identified.

MATERIAL AND METHODS

The present study is an experimental study in which the distribution of stress is investigated and compared in 4 maxillary implant-dependent overdentures by FEA method. In this study, the CBCT-Scan (NewTom VGi; Finland) of a 30-year-old patient that was available in the radiology department of Ahwaz Dental Faculty was used to prepare the Maxilla model. Maxilla data were imported to the Mimics ver. 8.1.1 for digitalization of images. After transferring CBCT-Scan data to the Mimics software, other changes were made to this data, which included deleting the patient's tooth data from the initial data. The entire maxilla structure and root site of the teeth was reconstructed with cortical bone and 2 mm soft gum tissue was considered on it. The maxillary bone structure was considered isotopic and homogeneous, while the elasticity coefficient was linearly assumed. The overdenture was constructed on the maxilla model that was obtained by a 3D printer of the existing model. It is essential to accurately measuring the implant for implant modeling. Hence, one specimen of SPI implant (Thommen Medical, Element, Switzerland) with a diameter of 4x12.5 mm was measured by the Coordinate Measuring Machine (CMM; Mitutoyo-America Corporation) and the dimensions required for modeling were used in solid works 2014. In this study, Ball, Bar-clip, Locator (ThommenMedical, Switzerland) attachments were used. Overdenture and attachments were initially measured by CMM and turned into digital images. The output file of the Mimics software was imported to the modeling software called Solid Works (SolidWorks® Office Premium 2007 SP, Corporation, Concord, MA, USA) to turn into a geometric model. Geometric modeling in Solid Work software was performed automatically based on the surface detail specified by the user. In the above model, implants were placed in the right second premolar, left and right canine and left central regions. One 4-mm long locator with housing with a diameter of 3.6 mm and a height of 2.3 mm was modeled. Also, ball with a width of 2.2 and a height of 4.3 mm and bar-clip with width and height of 2.2 and 3 mm respectively were modeled. In the Bar design, the abutment pattern was used and the outside part of the implant had height of 5.5 mm.

The next step in FEA modeling is to apply appropriate boundary conditions and loading. For this purpose, the geometric model was imported to ABAQUS / Standard software (Version 6.14/1, Pawtucket, IR) for finite analysis. ANSYS software was used to implement FEA modeling. The elements used in this study included SOLID187, CONTA174 and TARGE170, the first of which was used to generate the grid in the geometric model components and the latter two elements for the attachments. In the loading stage, the 100N static forces were applied vertically to the center of the first molar center and the stress distribution was demonstrated on the surface of all implant supporting attachments and bones based on computer graphic forms.

RESULTS AND DISCUSSION

Among the three different designs examined, the highest stress was observed in the vertical force (22.87 MPa) imposed on the implant in the right second premolar on the right overdenture supported by the locator implant. In the second place, the highest stress was

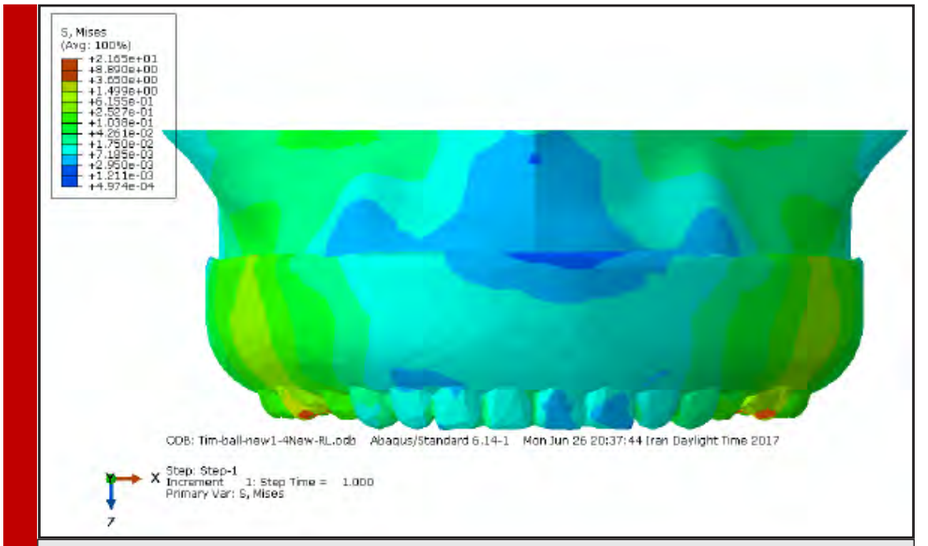

FIGURE 1. Distribution of stress caused by the bilateral force in ball attachment-based overdenture

observed on the Ball-retained overdenture in the right side force, imposed on the implant in the right second premolar corresponding to 12.88 MPa. The least stress among these three designs was observed in the bar-clip-retained overdenture design, the maximum stress on which is in the vertical force imposed on the right side to the right second premolar implant at the size of 7.486MPa. In the working side of the bone, the most stress was induced to the nearest implant of the same side. The results for the highest levels of stress in dense and sponge bones did not reach the final bone resorption in any of the treatment plans (Figures 1 to 4). Therefore, it can be concluded that overdenture based on the bar-clip implant with the least stress produced by the 100 N force, is the most suitable design, and the ball design has the least stress and is suitable in the second place (Tables 1 to 6).

In the working side of the bone, the most stress was imposed to the nearest implant of the same side. The results for the highest levels of stress in dense and

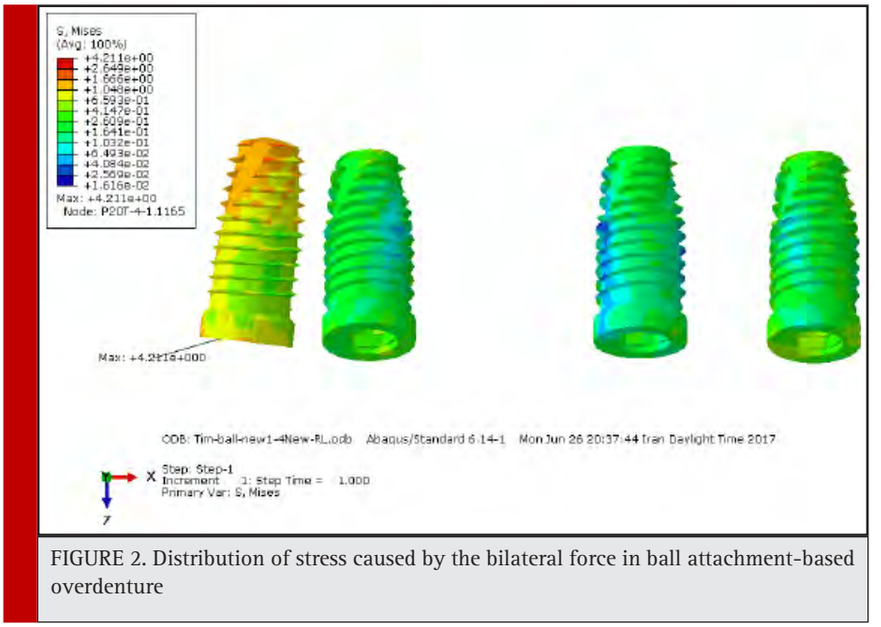

FIGURE 2. Distribution of stress caused by the bilateral force in ball attachment-based overdenture

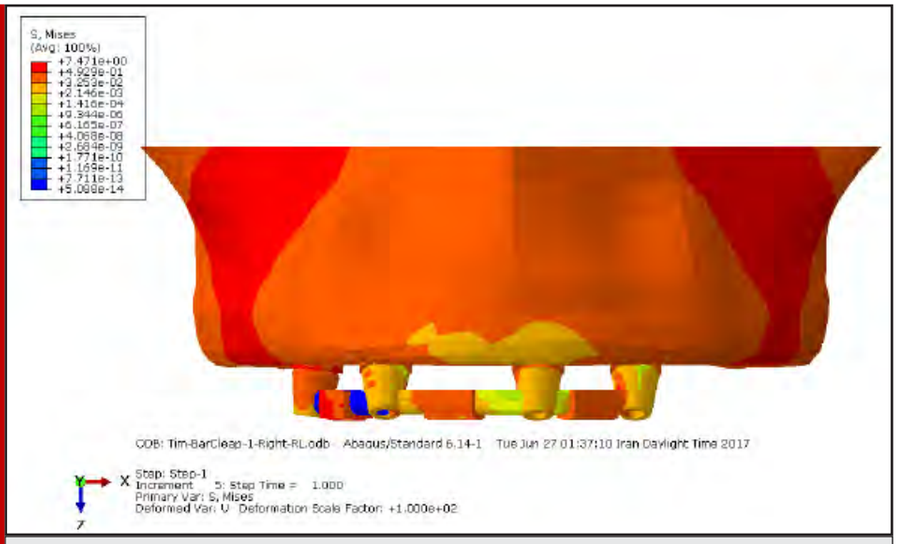

FIGURE 3. Distribution of stress caused by the bilateral force in bar-clip attachment-based overdenture

sponge bones did not reach the ultimate bone resistance in any of the treatment designs (Figures 1 to 4).

Among the three different attachment designs studied in this study, the highest bone stress was observed around the implant of the second right premolar area (working) where locator attachment was used. In the present study, the locator attachment was not evaluated using the FEA method. After the locator attachment, the highest stress was observed in the bone around the implant of the second premolar area (working) using the ball attachment and the result was consistent with the result of the study by Chun et al. with the difference that the vertical input force was 150 N was applied only bilaterally. In the present study, vertical forces were applied unilaterally and bilaterally with the highest concentration of stress in the working side of the bone near the nearest implant to the loading site in both cases.

According to the non-uniformity of force distribution obtained in the study of Mejer et al., the highest the stress was seen in the working side of the bone and

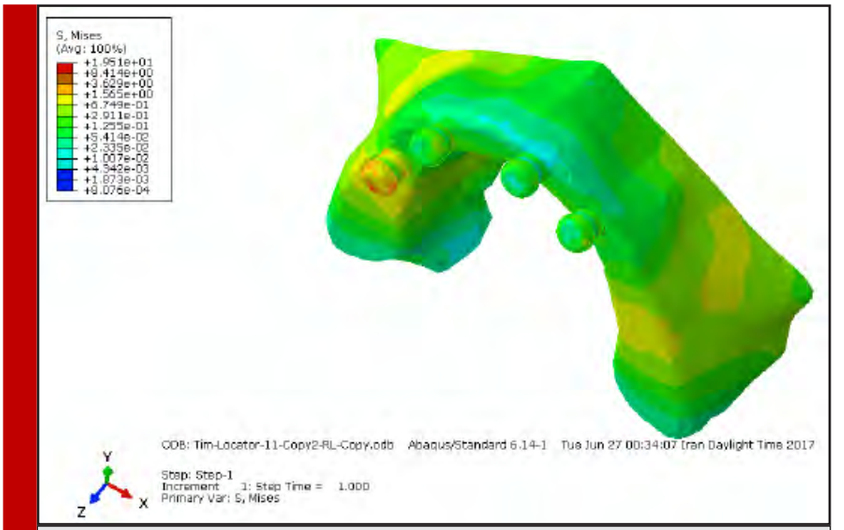

FIGURE 4. Distribution of stress caused by the bilateral force in locator attachment-based overdenture

Table 1. Maximum stress in implants in different treatment designs (Mpa) (bilateral force)

Left central	Tooth Left canin	Tooth right canin	Right second premolar	Treatment plan
0/01	0/674	0/674	1/56	Locator
0/0071	0/0071	0/0071	0/615	Ball
0/070	0/070	0/070	1/3	Bar-clip

Table 2. Maximum stress in implants in different treatment designs (MPa) (left force)

Left central	Tooth Left canin	Tooth right canin	Right second premolar	Treatment plan
0/017	0/017	0/00801	0/00801	Locator
0/075	0/2033	0/075	0/040	Ball
0/014	0/3239	0/014	0/075	Bar-clip

Table 3. Maximum stress in implants in different treatment designs (MPa) (right force)

Left central	Tooth Left canin	Tooth right canin	Right second premolar	Treatment plan
0/0012	0/063	0/168	0/4863	Locator
0/0031	0/127	0/127	0/3218	Ball
0/031	0/031	0/4863	1/202	Bar-clip

Table 4. Maximum stress in bone around implants in different treatment designs (Mpa) (bilateral force)

Left central	Tooth Left canin	Tooth right canin	Right second premolar	Treatment plan
0/067	0/067	0/067	22/87	Locator
0/0071	0/0071	0/0071	12/88	Ball
0/0078	0/0078	0/0078	7/486	Bar-clip

the nearest implant to the force insertion site, and the results confirms the consistency of both studies. According to the results, the lowest concentration of stress in the bone around the implant was observed when load-clip-based overdenture was used, which is inconsistent with the results obtained in the study of Menicucci et al. on two types of ball and bar attachments. They used a vertical 35N force on the mandibular overdenture, and the results showed that ball attachment tends to transmit less stress than the bar-clip attachment in the bone around the implant.

In the present study, the vertical force input was investigated in maxilla and the resulting difference requires a review of similar studies in this area. In a

Table 5. Maximum stress in the bone around the implants in different treatment designs (MPa) (left force)

Left central	Tooth Left canin	Tooth right canin	Right second premolar	Treatment plan
0/170	1/477	0/170	0/3626	Locator
0/548	0/3626	1/477	0/075	Ball
0/114	0/323	7/365	0/0403	Bar-clip

Table 6. Maximum stress in the bone around the implants in different treatment designs (MPa) (right force)

Left central	Tooth Left canin	Tooth right canin	Right second premolar	Treatment plan
0/168	7/486	0/450	22/87	Locator
0/809	0/809	0/809	12/88	Ball
0/0315	0/450	0/486	7/486	Bar-clip

previous study, locator, ball and bar-clip attachments, respectively, caused the highest amount of stress to the bone around the implant in maxilla. Valentim et al. obtained similar results by investigating Ball & Bar, Ball and Bar attachments in the mandible by applying a vertical force of 100 N and found that the highest stress was fed through the Ball type attachment to the bone around the implant. In the present study, the highest level of stress in the bone around the implant was concentrated in the implant neck region, which was completely consistent with the results of the previous studies. In the study of stress in metal parts in ball attachments, the stress concentration occurred in the cervical area of the attachment in the 5th tooth of the right implant area. The stress concentration for the bar-clip attachment was observed between the 5th and 3rd tooth right implants. In the locator attachment, the greatest stress in the housing area of the locator attachment was entered into the right side of the 5th tooth. Compared to the different treatment designs, the stress at the bar-clip attachments was higher than the other two. It is recommended to use the bar-clip treatment design in some cases where reducing stress in the bone around the implant is more important than overdenture stability and stress in the metal parts. If there is no necessity in these cases, you can use the Ball and Locator treatment designs as needed.

The greatest amount of bone stress in all treatment designs was concentrated in the cervical implants in the working side and a few upper threaded implants, and the stress rate didn't reach to the ultimate bone strength in any of the treatment designs, thus, it seems that bone resorption will not occur in any none of the treatment designs. In clinical situations where overdenture is expected to undergo lot of force, it is recommended to use the Bar-Clip treatment design because less stress is transmitted to the bone around the implant. The maximum stress induced in the implant-based overdenture model was observed in the locator attachment, and the implant overdenture supported by the Bar-clip attachment with less stress was the most appropriate design for the present study.

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In vitro anticariogenic activity of commercially existing anticavity tooth pastes and tooth powders against *Lactobacillus acidophilus* isolated from childhood caries

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ABSTRACT

Primary childhood caries constitute a burden for the dental profession and public health administrators, as many authors report that the situation in primary dentition has not improved over the last decades, impacting children's quality of life and future oral health. Biological as well as socio economic factors are related to the development of dental caries, and are both relevant in understanding the disease process and is the main reason the rural preschool children's are admitted to hospitals. The early stages of invasion infection caused by *Lactobacillus acidophilus* also play major role in this process. This study was carried out to access the invitro antibacterial potential of the different tooth paste and tooth powders available in local market against the isolated oral micro flora *Lactobacillus acidophilus* from hundred different samples (including tooth scraping and swabs) collected from various dental clinics and from rural preschool children with different age group ranging from 5-17yrs. A total of hundred isolates were selected and screened for their ability to produce antimicrobial substance. The antimicrobial activity of eleven different tooth pastes such as Pepsodent, Colgate advance, Meswak, Vicco, S.V Namboodiri, K.P. Namboodiri's, Babool, Dabur Red, Close up, Himalaya herbal complete care and Cheerio gel and four different tooth powders namely Herbal JM, Gopal, Pyorea and Injection were evaluated on cariogenic lactic acid bacteria. The antimicrobial properties of tooth paste were tested with cariogenic biofilm producing microbial strain *Lactobacillus acidophilus* using well diffusion method. Each tooth paste was tested at different concentrations (2%, 4%, 6%, 8%, 10% and 12%). This investigation showed that all dentifrices selected for the study were effective against the entire test organisms but to varying degrees. Pepsodent – Germi Check tooth paste gave a reading of 45 mm at 10% concentration of tooth paste as the zone of inhibition which was highest amongst all of the test dentifrices followed by Colgate advance dentifrice recorded a larger maximum zone of inhibition, measuring 40 mm compared to other tooth pastes. All other dentifrices showed the zone of inhibition to be between 20 mm to 37.5 mm in 10% concentration of tooth paste respectively. The data obtained from the complete investigation showed that tooth paste formulations have more effective active ingredients to control the oral micro flora whereas, herbal based products are equally effective as the other formulations but are not superior to them, whereas the tooth powder formulations were 100% less effective in controlling the oral flora.

KEY WORDS: ANTICARIOGENIC ACTIVITY TOOTH PASTES *LACTOBACILLUS ACIDOPHILUS*

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INTRODUCTION

WHO declared that deprived oral health and its related diseases may have dreadful effect on common health as well as eminence of life. Dental caries is an infectious, communicable disease resulting in destruction of tooth structure by acid forming bacteria found in dental plaque, an intraoral biofilm, in the presence of sugar. The infection results in loss of tooth minerals that begin on the outer surface of the tooth and can progress through the dentin to the pulp, ultimately compromising the vitality of the tooth. Various literature has proved that *Streptococcus mutans* and *Lactobacillus acidophilus* were the main causative agent for dental caries (Fitzgerald and Keyes, 1960). The early manifestation of the caries process is a small patch of demineralised enamel at the tooth surface, often hidden from sight in the fissures of teeth or in between the teeth. The destruction spreads into the softer, sensitive part of the tooth beneath the enamel (Yadav and Prakash 2017 and Tasie *et al.,* 2017).

These bacteria are strongly stimulated by sucrose and are known as the main organisms responsible for human tooth decay. Biofilm formation is a natural process in the oral environment, but needs to be controlled through regular brushing in order to prevent the development of caries and periodontal diseases. Most of the people use toothpaste without knowing their potential efficacy, bacteria form an important group of microorganisms found in both healthy and diseased mouths. There have been more than 300 types of bacteria found in the mouth; it's a very serious matter to select the effective toothpaste or powder to prevent teeth from microbial attack preventing the principle dental diseases like dental plaque, dental caries, gingivitis and periodontitis.

The purpose of oral hygiene using toothpaste is to reduce oral bacterial flora, mouth bacteria have been linked to plaque. Plaque is a complex biofilm found on the tooth surface that is a major cause of the development of dental caries (Benson *et al*., 2004). The accumulation and development of plaque depends upon the outcome of the interactions between the adhesiveness of plaque to the tooth surface and the physical shear forces which serve to dislodge and remove the plaque (Roberts *et al*., 2005). Tooth paste is classified as drug not as a cosmetic, different brands of tooth pastes and tooth powders contain effective antibacterial ingredients as broad spectrum antibacterial agents which effectively reduce oral bacteria and contribute to dental health (Regos *et al*., 1974). The common method for maintaining good oral hygiene is brushing the teeth with dentifrices that have antimicrobial properties and can prevent the degradation of tooth enamel (Vyas *et al*., 2017).

Tooth brushing with toothpaste is the most widely practiced form of oral hygiene in most countries (Pannuti *et al*., 2003). The success of any toothpaste, in part, lies on its ability to eliminate pathogenic oral micro flora. Fluoride dentifrices have been widely used all over the world and extensive research has established their abilities in terms of caries resistance (Itthagarum and Wei, 1996). A wide range of chemicals, mainly antimicrobial agents, have been added to tooth pastes in order to produce a direct inhibitory effect on plaque formation (Fine *et al*., 2006; Pannuti *et al*., 2003), clearly, most individuals find it difficult to maintain an effective level of plaque control and this is reflected in the levels of periodontal disease in the population. The addition of antimicrobial agents to tooth paste has been suggested as one possible method to improving the efficacy of mechanical tooth-cleaning procedures (Fine *et al*., 2006; Moran *et al*., 1988), aiding the control of dental plaque and preventing dental caries and periodontal diseases (Ozaki *et al*., 2006; White *et al*., 2006).

When these substances are added to oral products, they kill microorganisms by disrupting their cell walls and inhibiting their enzymatic activity. They prevent bacterial aggregation, slow multiplication and release endotoxins (Bou-Chacra *et al*., 2005; Ozaki *et al*., 2006). Our day begins with the tooth paste, hence the objective of this current study has made an effort to argument out the components of conventional tooth pastes should be as safe as those of herbal tooth pastes and able to maintain the good dental hygiene by use of tooth paste because dental care is one of the aspects of human sanitation, which unfortunately receive scanty attention to provide efficient protection against cariogenic microorganisms to enhance biofilm control, and prevent dental caries.

MATERIALS AND METHODS

Fifty different dental clinical samples from various dental clinics and from the patients having dental caries who come for the dental treatment of different age groups ranging from 5-17 years were collected with the help of an excavator and immediately transferred to 3 ml of saline solution in sterile glass vials. Information of patient's dental case history was also recorded along with his/her consent. After inoculation, vials were capped and sealed by Parafilm. The packed vials were brought to the laboratory immediately and kept in incubator at 37°C for 24 hours for bacterial enrichment. he cariogenic biofilm producing *Lactobacillus acidophilus* were screened, identified and purified by series of subculture on specific media such as Man Rogosa Sharpe agar and Nutrient agar were incubated aerobically at 37°C for 24 hours. The identification of all the microbes was confirmed by standard biochemical and staining methods (Aneja, 2003). All the pure cultures were stored and maintained in nutrient broth at 4˚C for further use.

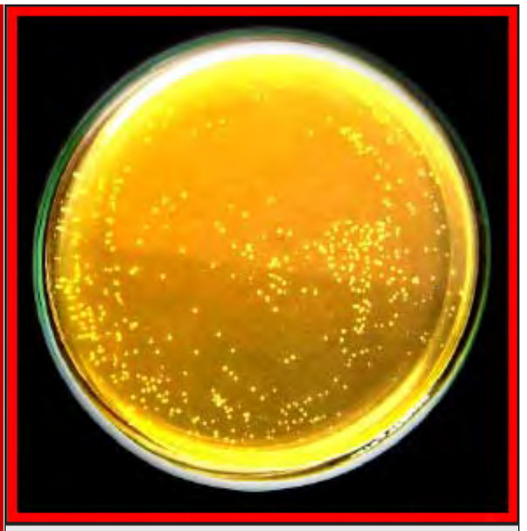

FIGURE 1. Colony of *Lactobacillus acidophilus* on Man Rogosa Sharpe agar

All leading conventional, herbal tooth paste and tooth powder brands were purchased from local market. The care was taken to procure the completely herbal (organic) toothpastes only. Fifteen samples namely Pepsodent, Colgate advance, Babool, Vicco, Dabur red, Herbal JM, Pyorea powder, Himalaya herbal complete care, S.V. Namboodiri's, K.P. Namboodiri, Gopal powder, Close up, Cherriogel, Meswak and Injection powder were collected.Different concentration of (2%, 4%, 6%, 8%, 10% and 12%) tooth pastes and tooth powder samples were diluted in sterile distilled water, shaken and kept as a stock solution. The distilled water employed as the negative control.

The antibacterial activity of the different concentrations: 2:100, 4:100, 6:100, 8:100, 10:100 and 12:100 (prepared by mixing 2g, 4g, 6g, 8g, 10g and 12g each of the toothpastes and tooth powders in 100 ml of sterile distilled water respectively) of the various tooth paste and tooth powder brands namely: Pepsodent, Colgate advance, Babool, Vicco, Dabur red, Herbal JM, Pyorea powder, Himalaya herbal complete care, S.V. Nambood-

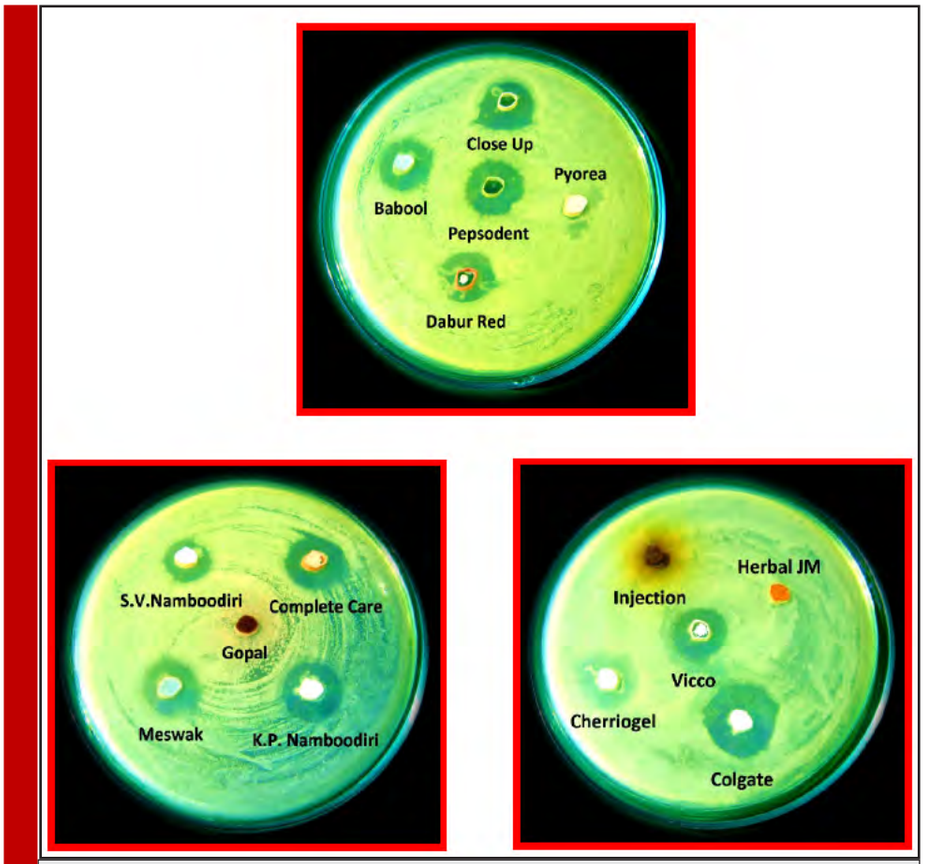

FIGURE 2. Anticariogenic activity of different dentifrices against *Lactobacillus acidophilus*

FIGURE 3. Dentifrices used in this study

iri's, K.P. Namboodiri, Gopal powder, Close up, Cherriogel, Meswak and Injection powder was determined by modified agar well diffusion method as described by Prasanth, (2011). In this method Muller Hinton agar plates were seeded with 0.5ml of 0.5 McFarland standards (approx., 10^8 cfu/ml) of cariogenic biofilm producing *Lactobacillus acidophilus*). The plates were allowed to solidify. A sterile 8mm cork-borer was used to cut one central and five wells at equidistance of the plates. 0.2ml of the tooth paste and tooth powder dilutions was inoculated to each of the five wells while the same amount of sterile distilled water was introduced in to the first well as control. The efficacy of anticariogenic tooth paste and tooth powder extracts against bacteria was compared with the broad spectrum antibiotics ampicillin, tetracycline and chloramphenicol (positive control). The same procedure was used for the broad spectrum antibiotics and the plates were incubated at 37˚C for 24hours. The antimicrobial activity was evaluated by measuring the diameters of zone of inhibition (in mm). An organism was interpreted as highly susceptible if the diameter of inhibition zones was more than 30 mm, intermediate if diameter was 25 to 30 mm and resistant was less than 25 mm.

RESULTS AND DISCUSSION

Amongst 100 clinical decay samples, 100 positive *Lactobacillus acidophilus* were isolated from different age group ranging from 5-17yrs. All the decay samples were tested on Man Rogosa Sharpe agar (MRS) for the isolation of *Lactobacillus acidophilus* and confirmed according to Bergy's manual of Bacteriology. Isolated cariogenic *Lactobacillus acidophilus* isolates were tested *invitro*

FIGURE 3. (*Continued*)

to determine anticariogenic activity of tooth paste and tooth powder by well diffusion method. There are different concentration (2%, 4%, 6%, 8%, 10% and 12%) of tooth paste and tooth powder were tested namely Pepsodent, Colgate advance, Babool, Vicco, Dabur red, Herbal JM, Pyorea powder, Himalaya herbal complete care, S.V. Namboodiri's, K.P. Namboodiri, Gopal powder, Close up, Cherriogel, Meswak and Injection powder. Totally 12 tooth pastes and 3 tooth powders were used to identify the potential efficacy of tooth pastes and tooth powders against the selected *Lactobacillus acidophilus*.

The results of antibacterial activity of different brands of anticavity tooth pastes and tooth powders against cariogenic bacteria are shown in Table 1 - 10. Among three brands, Brand – A Conventional tooth paste formulation include Colgate advance, Pepsodent, Close-up and Cherriogel was perceived maximum zone of inhibition (40, 47.5, 30 and 37.5 mm respectively) in 10% concentration followed by minimum zone of inhibition was scattered in 2% concentration of tooth paste.

In Brand – B, 7 dentifrices: Vicco, Dabur Red, Babool, Meswak, Himalaya herbal complete care, K.P. Namboodiri and S.V. Namboodiri were showed maximum activity (20mm, 31mm, 30mm, 31.5mm, 27.5mm, 30.5mm and 25 mm) in 10 % concentration of herbal based tooth paste followed by minimum zone of inhibition was dotted in 2% concentration of Vicco, Dabur Red, Babool, Meswak, Himalaya herbal complete care, K.P. Namboodiri and S.V. Namboodiri was significantly resistant against the tested cariogenic isolates get equally 0 mm zone of inhibition.

The findings from the present study the Brand – C include four diverse tooth powders namely Herbal JM, Injection, Gopal and Pyorea was notably degree of resistant against test pathogen *Lactobacillus acidophilus*. Awareness of this research was investigated to choice the best tooth paste, were using every day to reduce proliferation microorganisms in mouth as well as bad smell of mouth because of presenting microorganism in mouth and were found food between teeth .In the present study, three different brands of tooth pastes designated as A, B and C was tested for antibacterial activity against ten dental pathogens. All type of different tooth paste and tooth powder were found to be effective against 10 cariogenic pathogens to varying degree.

Tooth paste brand A (Type – Conventional) was significant in controlling the *Lactobacilli* infection from low concentration of 2% it exhibit significant degree of resistant against test pathogen showing there is no zone of inhibition observed in Strain No. 1 MTLACVG02 (Close up and Cherriogel), Strain No. 4 MTLACVG36 (Cherriogel), Strain No. 5 MTLACVG38 (Cherriogel), Strain No.10 MTLACVG100 (Close up) and increased with concentration showing 25 mm zone at 10 % in Strain No. MTLACVG100

Table 1. Antibacterial activity of tooth pastes and tooth powders against cariogenic strain MTLACVG02.

S. No.	Test Dentifrices	Zone of inhibition (mm) at different concentration of tooth paste and tooth powder					
		2%	4%	6%	8%	10%	12%
	Babool	0	14	15	17.5	19	15
	Vicco	0	10	12.5	14.5	17	0
	Dabur	0	15	16	20	22	0
	Herbal JM powder	0	0	0	0	0	0
	Pyorea powder	0	0	0	0	0	0
	Himalaya herbal complete care	0	12	15	15.5	25	0
	S.V. Namboodiri's	0	16	17.5	20	20	0
	Pepsodent	19.5	21	23	41.5	47.5	15
	K.P. Namboodiri	0	14	15	16.5	25	0
	Gopal powder	0	0	0	0	0	0
	Close up	0	14	15	18	30	0
	Meswak	0	12.5	13	17.5	31.5	13.5
	Injection powder	0	0	0	0	0	0
	Cherriogel	0	12.5	17	17.5	25	11
	Colgate advance	21	22.5	23	24	30	21

Table 2. Antibacterial activity of tooth pastes and tooth powders against cariogenic strain MTLACVG06.

S. No.	Test Dentifrices	Zone of inhibition (mm) at different concentration of tooth paste and tooth powder					
		2%	4%	6%	8%	10%	12%
	Babool	15	15	15	16.5	20	15
	Vicco	10	11	13	13	15	12
	Dabur	14.5	15	15	16	20	14.5
	Herbal JM powder	0	0	0	0	0	0
	Pyorea powder	0	0	0	0	0	0
	Himalaya herbal complete care	12	15	19	20	27.5	11.5
	S.V. Namboodiri's	12	14	15	20	22.5	11.5
	Pepsodent	18	21	28.5	30	35	15
	K.P. Namboodiri	11	12	13.5	14.5	15.5	14
	Gopal powder	0	0	0	0	0	0
	Close up	14	15	15.5	19	27.5	11
	Meswak	10	10	13	13	21.5	13
	Injection powder	0	0	0	0	0	0
	Cherriogel	11	13	13	20	25	13
	Colgate advance	21	22	26.5	29	36.5	18

Table 3. Antibacterial activity of tooth pastes and tooth powders against cariogenic strain MTLACVG17.

S. No.	Test Dentifrices	Zone of inhibition (mm) at different concentration of tooth paste and tooth powder					
		2%	4%	6%	8%	10%	12%
	Babool	12	13	14.5	15	17.5	12
	Vicco	10	12	12.5	15	17.5	12
	Dabur	10	12	16	16	30	10.5
	Herbal JM powder	0	0	0	0	0	0
	Pyorea powder	0	0	0	0	0	0
	Himalaya herbal complete care	12	15	15.5	18	18.5	18
	S.V. Namboodiri's	12	14.5	15	18.5	23	8
	Pepsodent	15	17.5	25	29.5	31	13.5
	K.P. Namboodiri	12	12	12	15	15	10.5
	Gopal powder	0	0	0	0	0	0
	Close up	13	15	15	16	16	10
	Meswak	9	10	14.5	15	17.5	15.5
	Injection powder	0	0	0	0	0	0
	Cherriogel	13	14.5	15	16.5	20	9
	Colgate advance	16.5	20	23.5	24.5	27.5	11.5

Table 4. Antibacterial activity of tooth pastes and tooth powders against cariogenic strain MTLACVG36.

S. No.	Test Dentifrices	Zone of inhibition (mm) at different concentration of tooth paste and tooth powder					
		2%	4%	6%	8%	10%	12%
	Babool	0	13	19.5	20	20	0
	Vicco	9	10	13	15.5	16	0
	Dabur	10	14	17.5	21	30	0
	Herbal JM powder	0	0	0	0	0	0
	Pyorea powder	0	0	0	0	0	0
	Himalaya herbal complete care	0	18	19	19.5	22.5	0
	S.V. Namboodiri's	0	14	16.5	18.5	22.5	0
	Pepsodent	17	23.5	25	27.5	30	0
	K.P. Namboodiri	11	12.5	14	15.5	16	0
	Gopal powder	0	0	0	0	0	0
	Close up	15	16.5	17	20	30	0
	Meswak	11	12	15	18	20	0
	Injection powder	0	0	0	0	0	0
	Cherriogel	0	14	15	20	37.5	0
	Colgate advance	18	18	27.5	27.5	40	16

Table 5. Antibacterial activity of tooth pastes and tooth powders against cariogenic strain MTLACVG38.

S. No.	Test Dentifrices	Zone of inhibition (mm) at different concentration of tooth paste and tooth powder					
		2%	4%	6%	8%	10%	12%
	Babool	10	15	16.5	19	22	13.5
	Vicco	13	13	14	15	18	12
	Dabur	13.5	14	15	17	20	15
	Herbal JM powder	0	0	0	0	0	0
	Pyorea powder	0	0	0	0	0	0
	Himalaya herbal complete care	12	15.5	16	20	24	14.5
	S.V. Namboodiri's	0	15	17.5	18	20	12.5
	Pepsodent	23	23	33.5	34.5	41.5	14
	K.P. Namboodiri	0	11	14	15	25	13
	Gopal powder	0	0	0	0	0	0
	Close up	14	15.5	19.5	20	22	13.5
	Meswak	12	13	13.5	14	17.5	13.5
	Injection powder	0	0	0	0	0	0
	Cherriogel	0	13.5	16.5	20	22	13.5
	Colgate advance	20.5	22.5	25	25	30	18

Table 6. Antibacterial activity of tooth pastes and tooth powders against cariogenic strain MTLACVG75.

S. No.	Test Dentifrices	Zone of inhibition (mm) at different concentration of tooth paste and tooth powder					
		2%	4%	6%	8%	10%	12%
	Babool	12.5	14	14	18.5	30	14.5
	Vicco	10	13	13	14	15	15
	Dabur	13	15	15	19	31	15
	Herbal JM powder	0	0	0	0	0	0
	Pyorea powder	0	0	0	0	0	0
	Himalaya herbal complete care	11	15	15	17.5	22.5	16
	S.V. Namboodiri's	9	14.5	15	17.5	20	14.5
	Pepsodent	17.5	20	24	25.5	39	12.5
	K.P. Namboodiri	11	13	14	15	15	13
	Gopal powder	0	0	0	0	0	0
	Close up	13	15	15	19	32.5	11
	Meswak	10	12	12.5	15	16	13
	Injection powder	0	0	0	0	0	0
	Cherriogel	12	14	15	20	20	15
	Colgate advance	18	21	25	25.5	30	15

Table 7. Antibacterial activity of tooth pastes and tooth powders against cariogenic strain MTLACVG85.

S. No.	Test Dentifrices	Zone of inhibition (mm) at different concentration of tooth paste and tooth powder					
		2%	4%	6%	8%	10%	12%
	Babool	14.5	17	19	20	25	11
	Vicco	8	14	14	15	17	12
	Dabur	12	14	15.5	17	19	14.5
	Herbal JM powder	0	0	0	0	0	0
	Pyorea powder	0	0	0	0	0	0
	Himalaya herbal complete care	13	18.5	19	20	22	14.5
	S.V. Namboodiri's	11	15	17	17	17.5	14.5
	Pepsodent	20	25.5	26	27.5	30	12
	K.P. Namboodiri	12	14	16.5	20	20	12.5
	Gopal powder	0	0	0	0	0	0
	Close up	13	15	15.5	16.5	17	14.5
	Meswak	0	13	13	13.5	17.5	14
	Injection powder	0	0	0	0	0	0
	Cherriogel	9	14	16.5	20	20	13.5
	Colgate advance	17	19.5	25	26.5	27	20

Table 8. Antibacterial activity of tooth pastes and tooth powders against cariogenic strain MTLACVG95.

S. No.	Test Dentifrices	Zone of inhibition (mm) at different concentration of tooth paste and tooth powder					
		2%	4%	6%	8%	10%	12%
	Babool	14	15	16	20	24.5	15.5
	Vicco	9	10	14	14	13.5	15
	Dabur	10	18.5	20	20	20	18
	Herbal JM powder	0	0	0	0	0	0
	Pyorea powder	0	0	0	0	0	0
	Himalaya herbal complete care	13	15	16.5	19	20	0
	S.V. Namboodiri's	15	17.5	18.5	19	25	15.5
	Pepsodent	19	20	22.5	27.5	28	14.5
	K.P. Namboodiri	10	12.5	15.5	17.5	18	14.5
	Gopal powder	0	0	0	0	0	0
	Close up	13	14	17	17	20	13.5
	Meswak	7	12.5	15	16	17	14.5
	Injection powder	0	0	0	0	0	0
	Cherriogel	9	14	16.5	20	20	13.5
	Colgate advance	17	19.5	25	26.5	27	20

Table 9. Antibacterial activity of tooth pastes and tooth powders against cariogenic strain MTLACVG97.

S. No.	Test Dentifrices	Zone of inhibition (mm) at different concentration of tooth paste and tooth powder					
		2%	4%	6%	8%	10%	12%
	Babool	13	15	17	18	19	14.5
	Vicco	7	12.5	15	16	16.5	13.5
	Dabur	14	17.5	18.5	20	23	15.5
	Herbal JM powder	0	0	0	0	0	0
	Pyorea powder	0	0	0	0	0	0
	Himalaya herbal complete care	18	19	19.5	20	22	22
	S.V. Namboodiri's	14.5	15	16	16.5	18	13.5
	Pepsodent	19	21	22.5	22.5	30.5	13.5
	K.P. Namboodiri	19	21	22.5	22.5	30.5	13.5
	Gopal powder	0	0	0	0	0	0
	Close up	12	15	16.5	18	19	20
	Meswak	11	13	15	17.5	17.5	15
	Injection powder	0	0	0	0	0	0
	Cherriogel	9	14.5	20	20.5	22.5	15
	Colgate advance	0	18	25	35	40	16

Table 10. Antibacterial activity of tooth pastes and tooth powders against cariogenic strain MTLACVG100.

S. No.	Test Dentifrices	Zone of inhibition (mm) at different concentration of tooth paste and tooth powder					
		2%	4%	6%	8%	10%	12%
	Babool	0	13	14	20	22.5	0
	Vicco	0	10	13	13	20	0
	Dabur	0	12	15	17.5	24	0
	Herbal JM powder	0	0	0	0	0	0
	Pyorea powder	0	0	0	0	0	0
	Himalaya herbal complete care	15.5	0	15	16.5	17	36.5
	S.V. Namboodiri's	0	15	15	19	25	0
	Pepsodent	25	25	25.5	35.5	45	15
	K.P. Namboodiri	0	12	15	15	17	0
	Gopal powder	0	0	0	0	0	0
	Close up	0	15	15.5	20	20	0
	Meswak	0	12.5	13	16.5	18	0
	Injection powder	0	0	0	0	0	0
	Cherriogel	13.5	15	20	20	21	0
	Colgate advance	20	24	24	32	15	0

Table 11. Tooth paste brands used in this study

S. No	Brand	Type	Tooth Paste
1.	Brand – A	Conventional	Colgate advance, Pepsodent germi check, Close up, Cherriogel.
2.	Brand – B	Herbal	Vicco, Dabur Red, Babool, Meswak, Himalaya herbal complete care, K.P. Namboodiri and S.V. Namboodiri.
3.	Brand - C	Powder	Gopal, Herbal JM, Pyorea and Injection

somewhat similar with dissimilar results was obtained in case of *Lactobacillus* spp., this results are in accordance with the results of (Sohail and Khan, 2013) they stated that some tooth paste shows better antibacterial activity against the flora and activity of tooth paste because of active ingredients such as fluoride.

The results regarding the brand B (Type – Herbal) is Herbal based products and exhibited least effectiveness as compared to other test formulations. The decay pathogen shows maximum zone of inhibition against ten cariogenic *Lactobacillus acidophilus* it exhibit better results was shown in 8% and 10% concentration rather than the lowest concentration (2%, 4% and 6%). This may be due to the ingredients present, the herbal formulation studied appeared to be equally effective as the fluoride formulations but not superior to them. Almas *et al*., (2001) reported that the antimicrobial activity of the herbs may be due to the presence of secondary metabolites such as alkaloids, flavonoids, polyphenols and lectins. Using natural medicines to cure various diseases so, hence herbal medicine had made significant contribution to modern medical practice.

Formulation brand C (Type – Tooth powder) include some dentifrices are listed in (Table 11). Among 2% - 10% concentration of tooth powders are strongly exhibit significant degree of resistant against ten cariogenic isolates (MTLACVG02, MTLACVG06, MTLACVG17, MTLACVG36, MTLACVG38, MTLACVG75, MTLACVG85, MTLACVG95, MTLACVG97 and MTLACVG100) it shows there is no zone of inhibition observed in all cariogenic isolates. Similarly, Sohail and Khan, (2013) also stated that tooth powder formulation is less effective to control the oral microorganisms compare than other conventional and herbal tooth paste formulations. The data obtained from the complete investigation it had shown that tooth paste formulations having more effective active ingredients to control the oral micro flora whereas, herbal based products are equally effective as the other formulations but not superior to them, whereas the tooth powder formulations was 100% less effective in controlling the oral flora.

CONCLUSION

The present study demonstrated that the antibacterial properties of fifteen dentifrices used against the dental pathogen and concluded that conventional tooth paste formulation (Brand – A) has promising anticariogenic effects compare than other formulations (Brand – B and Brand – C) to some extent to benefit anti plaque action. Further study is needed to determine the bioactive compounds which are responsible for this anticariogenic activity.

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Comparison of the effects of IPS e.max heat press layered and feldspathic porcelain on natural enamel tooth wear

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ABSTRACT

In an ideal situation, the tooth wear of a restorative material should be similar to dental enamel. Currently, All-ceramic restorations are widely used; But Enamel wear is among the main disadvantages of ceramic restorations. Thus, in this study compared the Effect of IPS e.max heat press layered and Feldspathic Porcelain on natural enamel Tooth Wear.In this study, 20 samples were prepared from normal human teeth. The natural teeth were photographed by a stereomicroscope in a fixed position and the distance from the cusp tip to a reference point was measured. Next, 10 teeth opposed IPS e.max heat press layered and the remaining 10 opposed Feldspathic Porcelain in a chewing simulator and subjected to 120,000 masticatory cycles. The teeth were photographed again and the greatest difference between the before and after values was recorded. Finally data were analyzed by Independent samples T-test. The mean extent of wear on the restoration layered IPS e.max heat press and Feldspathic Porcelain were not significant differences (p-value= 0.118); although the average wear in Feldspathic Porcelain was less than layered IPS e.max heat press. the distance from the cusp tip to a reference point in layered IPS e.max heat Press was significantly difference between before and after intervention (p-value<0.001). Also, the distance from the cusp tip to a reference point in FeldspathicPorcelain was significantly difference between before and after intervention (p-value<0.001). According to the results obtained in vitro study, the mean extent of wear on the restoration layered IPS e.max heat press and Feldspathic Porcelain were not significant differences. Also, it is recommended to have a closer look at the factors involved in tooth enamel wear in future studies.

KEY WORDS: FELDSPATHIC PORCELAIN, IPS E.MAX HEAT PRESS LAYERED, ENAMEL TOOTH WEAR

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INTRODUCTION

Replacement of missing teeth or repair a part of a tooth has long been considered by dentists, Currently using a variety of ceramic restorations has largely resolved this problem; Ceramic restorations have advantages including lack of fracture, lack of blanching and high strength are also by considering the aesthetic, ease of use and color similar to natural tooth using these restorations have increased daily. These restorations due to the lack of metal in their structure (metal component can cause problems such as chemical toxicity, kerogen, change the color of gums and creating allergic reactions to some metals such as nickel) are preferred compared with coated of metal - ceramic (Jung 2010) and Rosenstiel, 2015, Murillo-Gómez, 2017 and (Montazerian, 2017).

There are also disadvantages in addition to the top benefits of these restorations that the most important of them is front tooth enamel erosion (Shillingburg, 2013). Erosion is defined as damage to the tooth surface or losing volume of teeth by direct contact with the teeth or other materials. In fact, erosion is a physiological phenomenon that is happening naturally always mechanically or chemically (DeGee, 1986). The sharp rise of dental erosion can cause to loss of centric contacts, vertical elevation change, Change in Functional paths during chewing and fusion muscle fatigue (DeLong, 1989) and (Gallegos, 1988). Dental restorations change the natural erosion rate of front teeth if have had different erosion features (Sulong, 1990). So erosion between tooth and its front restoration should be always considered as important factor along with strength and beauty to selecting restorative matter (Seghi, 1991).Considering this, various methods have been tested to improve and reduce the erosion of the front teeth in ceramicrestorations; Among these methods, reinforced ceramics with alumina oxide crystals, Lusaite, lithium DE silicate and zirconia (Komine, 2004) and (Barath, 2003).

Given the importance of the front dental erosion by ceramic restoration, in this study, two restorations of the Feld spathic porcelain and layered IPS e.max heat press ceramic were evaluated and compared in terms of the front side tooth enamel. The reasons for choosing these two restorations can be reffered to improve the ceramic properties and porcelain bonding systems that has been caused to beauty and good performance of feldspathic porcelain (Vieira, 2004) and IPS e.max Press makers claim to improve physical and translucency properties, lower surface hardness low concentrations of crystalline phase and a smaller sizes of crystals at different stages of cooking Empress 2 and revise in formulation of the this kind of ceramic to obtaining high quality (Newsome 2014) and (Guess 2011).

Of course in laboratory studies that have been done in this area, a significant difference has not been specified in phase composition and the bending strength between IPS e.max Press and Empress 2 (Guazzato, 2004) and (Albakry 2003). IPS e.max Press in monolithic mode is used for inlays, onlays, full coverage of crown... (Guess 2011). In times of short and medium term have shown in reviewing these performances that IPS e.max Press restorations have good performance: Using this restoration in onlays in the 3-year period, the efficiency of 100% (Guess 2009) and the crown in the 3-year period has had the performance of 96.6% (Etman, 2008). Despite this high performance, high erosion possibility of this restoration on the tooth enamel can be considered as one of the major disadvantages (Esquivel-Upshaw 2012). Also, amount of natural tooth erosion against the layered IPS-emax heat press and feldspathic porcelain were discussed due to lower researches and lack of consensus on the abrasive effect of these ceramics and the need for further studies in this area.

MATERIALS AND METHODS

This study was done as In vitro and in Reference Laboratory and Research Center University of Medical Sciences in Tehran. The study population was healthy teeth recently extracted and without premolar decay of human maxillary. Easy non-probability sampling method and sample size were obtained from the results of previous studies (Ahmadzadeh, 2014) and by considering α=0.05, and power of 1-β = 80%, at least 20 teeth. Trend of running in this study was in this way that in each group 10 samples were prepared with dimensions of 10 × 10 × 10 mm. in IPS-emax heat press group in order to build cylinders of a block with cross-section dimensions 10 × 10 × 10 is molded with incremental silicone impression material and then wax blocks were prepared by melting the inlay10 wax. Then these wax blocks, were done sprue, cylindering and were cast by vita ceramic Inge. Specific cylinder device: Chewing simulator (CS-4.2 S / N: A100220128SM01) was used to cylindering (Figure 1).

Cylinder was heated to 800 ° C under to evaporate and remove the wax pattern. Vita ceramic ingot was placed within the cylinder by tongs and cylinder was placed in specific furnaces. After warming oven to temperature of 920 ° C, ceramic melted slowly and in vacuum was injected into the cylinder.Sprue cut off and the samples were sandblasted by 2-1 times of aluminum oxide after cooling cylinder. Then the blocks were drool with powder and liquid of VitaAkzent Plus .wax blocks was used to make Feldspathic porcelain with dimensions 10 × 10 × 8 mm has been molded with an incremental silicone impression material and then wax blocks were prepared by melting the inlay10 wax. Then these blocks were done sprue and cylindered and were cast by Vero bond (vita mark) alloy.

Porcelaining was done with VMK master (vita mark) porcelain by thickness of 2mm and external dimensions were measured by a gauge after cutting the sprue and sandblasting and grinding alloy with pink and white stone. Premolar healthy natural teeth of 4 human maxillary as well as were selected to the total number of samples and were stored in distilled water until erosion time. all samples were mounted by Survivor in a plastic mold in the shape of a half-cylinder in-hardening resin (Ivoclarvivadent) before testing. Then the photos were taken of dental teeth in Razi Metallurgical Research Center (Razi Metallurgical Research Center) by stereomicroscope (VEGA II TESCAN) in fixed position and a fixed place was determined for each sample. Distance from the cusps tip to the desired location was measured by the Motic Image Plus software. Then natural teeth were placed in the front samples of feldspathic porcelain and layered ips-emax heat press in chewing simulator device. The samples were immersed in distilled water at all times of erosion. After the erosion test, the photos were taken of samples by stereomicroscope again in the same previous position and due to mentioned method the measurement was done for each cusp again; the difference between the two amounts was recorded and the highest was recorded per micrometer. Data with SPSS software version 18 and by using statistical analysis Independent samples T test were used for statistical analysis. The significant level of 0.05 was considered in this study.

RESULTS AND DISCUSSION

In this study, with the help of Shapiro-Wilk test it was determined that the data are followed from a normal distribution (0.05 <p-value). Means of erosion rate between the two materials of layered IPS e.max heat press and Feldspathic Porcelain was evaluated by independent samples t-test and erosion difference between the two restoration was not statistically significant (p-value=0.118) although the mean of erosion rate in Feldspathic Porcelain was less than layered IPS e.max heat press (table 1).

As it is shown in Table 1; paired t-test showed that the difference between the mean difference of the vertical height of the tip of the buccal cusps to central fossa in tooth surface in IPS e.max heat press between before and after the intervention (0.001> p-value) and in Feldspathic Porcelain between before and after the intervention (0.001> p-value) was significant.

DISCUSSION

Ceramic materials made with respect to stiffness and strength of them are usually resistant to erosion, but erosion tighter than usual their front teeth is what is made problem in this type of restorations (Heintze, 2008). In the present study, there was no statistically significant difference between the mean of erosion rate in two restorations of layered IPS e.max heat press and Feldspathic Porcelain (0.118 = p-value), although mean of erosion rate in Feldspathic Porcelain somewhat was lower than layered IPS e.max the heat press.

In a study by Tian et al. (2013) that the erosion rate of two groups of Porcelain Ceramics, and layered IPS e.max heat press were studied, the results showed that the erosion rate on in the layered IPS e.max heat press ceramic has been much more than Porcelain (Tian, B.M 2013) which is inconsistent with results of this study. In a study of Esquivel-Upshaw the rate of Front tooth

Table 1. Mean and standard deviation the amount of wear before and after treatment in two different materials

Wear rate		*p*-value[a]	The vertical height of the buccal cusp tip of the tooth surface after the intervention of the central Tafusay		The vertical height of the buccal cusp tip of the tooth surface before the intervention of the central Tafusay		
Standard deviation	Average		Standard deviation	Average	Standard deviation	Average	
0/0673	0/6849	<0/001	0/3506	1/9141	0/3482	2/5990	layered IPS e.max heat press
0/1731	0/5859	<0/001	0/4596	1/6656	0/4769	2/2515	Feldspathic Porcelain
0/118			0/191		0/079		*p*-value[b]

[a]comparison before and after the intervention (paired t test)
[b]comparison between the two materials (independent samples t-test)

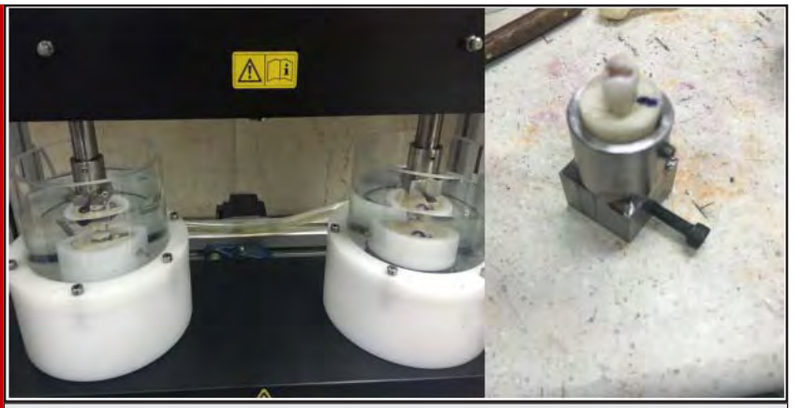

FIGURE 1. A prepared sample and chewing simulator

enamel erosion has been reported by restoration of IPS e.max heat press µm 88/3 (Esquivel-Upshaw 2012). in study of Etman, et al that also studied the erosion of Front tooth enamel in three types of ceramic restorations, the results showed that after two years of following the erosion mean created by Allceram Procera, µm 261, has been for IPS e.max heat press µm 215 and metal ceramics µm156 that this difference was statistically significant (Etman, 2008).

In this study, Means of vertical height of the tip of the buccal cusps to central fossa in tooth surface after the intervention there was no significant difference between the two materials (p-value=0.191); But vertical height means of the tip of the buccal cusps to central fossa tooth surface on the material of layered IPS e.max heat press there was a statistically significant difference between before and after the intervention (0.001> p-value) and in Feldspathic Porcelain between before and after the intervention (0.001> p -value). It is clear that the significant erosion has occurred with regard to these results. But the erosion differences have not shown obvious difference between two restorations. Heintze et al. by examining erosion rate of front ceramic teeth and factors affecting on it, have recognized that factors such as the shape of the samples, surface treatment, enamel thickness, type of milling device and its geometry shape, grains size and crystal size are effective in erosion (Heintze, 2008).

In fact, the erosion is related to the friction created between the two levels, so it can be concluded that surface properties and conducted interaction will have important impact by contact in the two levels. Smooth surfaces have less resistance and therefore less erosion compared to uneven surfaces; the erosion rate of them on front teeth will be also more whatever the surface roughness of restorative materials to be more. For this reason, it has always tried to maintain the strength of the restoration; its surface roughness is reduced as much as possible. According to studies conducted, the surface roughness is for feldspathic porcelains of MPa 6560 and for IPS e.max Press, MPa 5800 that due to the proximity of value of this variable in two restorations examined, lake of difference in Front tooth erosion is also justified (Al-Shehri 2002).

Another important factor in front tooth erosion fracture toughness. All ceramic materials that have been used in dentistry so far, has been with much lower fracture toughness than metals. According to studies for fracture toughness of IPS e.max Press MPam 1/22/ 1- 4/3 (14) and for feldspathic porcelain on range of MPam 1/290/0 up to be 1.56 (23), as well as natural tooth enamel fracture toughness has been reported in the range of MPam 1/237/1-7/0 (Heintze, S.D 2008) and natural teeth dentin has been reported in MPam 1/208/3 (24), that compared with metals have less fracture toughness between 20 and 100 times. Lee et al in New Zealand during a study have shown that gold brigade 3 compared to IPS e.max Press has less abrasive and friction on the tooth enamel and can be seen significant cracks in front enamel surface of samples of IPSe.max Press examples in comparison with gold brigade 3 (Lee 2012). Lee and et al differences in fracture toughness in different materials know as one of the Justifier factor; so that during the action and the inclusion of occlusal forces on the surface of the restoration are with micro fracture and lead to bumps and abuses such as inclusion Crystalline that are going off the surface of the material; as result leads to accumulation of a lot of pressure on enamel and troughs Gauging. In addition dug particles can act like an Abrasive and to create three-dimensional erosion; So it can be expected that less erosion is created in front teeth in the restorations with higher fracture toughness (Anusavice, 2013). Given that in terms of fracture toughness as well as there was no significant differences between two

restorations evaluated in this study, so the same Front tooth erosion is justifiable.

CONCLUSION

According to the results obtained in present experimental study, the means of erosion rate in two restorations of layered IPS e.max heat press and Feldspathic Porcelain has no statistically significant difference with each other, although mean of erosion rate in the Feldspathic Porcelain has been somewhat lower than layered IPS e.max heat the press; The results showed that the erosion on both the restorations after intervention has been significant compared to before intervention; As a result, it can be concluded that these two restorations causes to significant erosion of front tooth enamel, but have no significant difference with each other.

SUGGESTIONS

It is suggested that in future research erosion caused be evaluated by natural tooth enamel and other high applicable restorations and also be compared by two restorations of layered IPS e.max heat press and Feldspathic Porcelain. It is also possible to design and carry out long-term laboratory studies of this kind, to provide the information necessary to assess the relationship between laboratory and clinical erosion data.

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Ameliorative effect of salicylic acid in salinity stressed *Pisum sativum* by improving growth parameters, activating photosynthesis and enhancing antioxidant defense system

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ABSTRACT

Salt stress unfavorably disturbs the physiological processes and morphological organization of plants that limit plant growth and development. Salicylic acid (SA) is an important signal molecule, also acts as growth regulator that alleviates the hostile impact of salinity on plants. *Pisum sativum* L. (Fabaceae) is a salt-sensitive plant. This study was conducted to to explore the regulating mechanism of exogenous SA on the germination,growth, relative water content, chlorophyll content, ROS concentrations, and antioxidant activities of *Pisum sativum* sample under 50, 100 and 150 mM NaCl conditions. *Pisum sativum* showed reduced growth rate, dry weight, decreased photosynthetic Pigments, alteration in nutritional contents under 50 and 100 mM NaCl level of salinity. Severity of adverse effect was maximum at 150 mM of NaCl. However, presoaking application of SA efficiently retains the growth parameters, photosynthetic efficiency, and improved antioxidant defense system. However, at higher salinity levels i.e. at 150 mM NaCl saline condition, there was no noteworthy variance in the mitigation in terms of growth and other physiological responses were observed. We demonstrate that application of SA can meritoriously neutralize the adverse effect of moderate saline conditions on growth and development of *Pisum sativum*.

KEY WORDS: SALICYLIC ACID, SALT STRESS, ROS, ANTIOXIDANTS, PHOTOSYNTHESIS

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INTRODUCTION

Being sessile in nature and increased anthropogenic activities in the advanced era, plant exposed to innumerable abiotic stresses, such as heat cold stress, salinity, heavy metals, ultraviolet radiation, nutrient changes (Khan and Khan, 2013). Under natural conditions, salinity has a multiple effect on plant growth by reducing water absorption, creating ion imbalance or turbulence that leads to plant toxicity (Roussos et al., 2007). This negatively affects growth parameter *viz* germination efficiency, leaf area, length and root and shoot dry weight, protein synthesis, photosynthesis and chlorophyll, lipid breakdown, reactive oxygen species formation (Munns, 2005; Parida and Das, 2005). Over-production of ROS caused and declining photosynthesis (Gunes et.al., 2007; Steduto et al 2000). To readdress the loss due to ROS generation, plants have an effective defense system composed of both, enzymatic (SOD, CAT, POD) and non-enzymatic (Proline, Phenol contents, carotenoids, and tocopherol) and antioxidants systems. However, plants showed different response to salinity according to the plant tolerance capability and developmental stage. The treatment with 150 mM NaCl level of salinity enhanced about 72% of POD activity in salt-tolerant cotton (Gossett et al.,1994). Thus, it is important to enhance the salinity resistance of plants and endeavor different compound to reduce plant stress.

SA is a phenolic growth regulator that assumes conspicuous and expanded role in biochemical and physiological reaction to abiotic stress (Hayat et al. 2012).It has been also revealed that exogenously applications of SA can significantly enhanced overall growth of plant under both salinity and non-salinity by adjusting antioxidants scavenging system (Ismail 2013). Furthermore, SA also reduced the destructive effects of several abiotic stresses by regulating proline concentration and other osmolytes production (Pirasteh- Anosheh et al. 2014; Chandrakar et al. 2016 Ma et.al, 2017).

However, confirmation regarding the lessening of salinity stress by exogenous SA is slightly questioned. Arfan et al. (2007) and Li et al. (2014), recounted that spraying SA could balance direct salt stress actuated development restraint, while no change happened at high convergences of salt stress. Therefore, the action of SA to suppress salt stress on concentration and plant species needto be further clarified. Pea (*Pisum sativum* L.) second important leguminous crop belongs to family Fabaceae, grown throughout the world in winter to early summer, used both in human nutrition and as fodder. The nutritional value of pea plant cannot be neglected as it is an important source of major biomolecules protein, carbohydrate (Hussein et al; 2006), water-soluble fibers, vitamins (vitamin B1), antioxidants (Mukerji 2004) calcium, phosphorus, fibers, minerals and lutein with a small quantity of iron. It also contains isoflavones which reduced the risk of cancer. It can be grown on a different soil texture but unfavorably influenced by different abiotic burdens, for example, soil corrosiveness, aluminum toxicity and salinity stress. Field pea is extremely sensitive to salinity stress. Regrettably, insufficient studies are available on the growth and development of *P. sativum* under salinity stress. Additionally, the defensive role of phytohormones especially role of SA in ameliorating the detrimental effect of salt stress on *P. sativum* to increase its salt tolerance is mystical.

The aim of present study was to elucidate the alleviating effect of SA in *P. sativum* plants under different concentration of salt treatment by studying various morphological, physiological and biochemical parameters such as biomass yield, total protein and sugar, relative water and proline content. The analysis of antioxidant activities were also done to analyzed ROS generation.

MATERIALS AND METHODS

PLANT MATERIALS AND GROWTH CONDITIONS

The *P. sativum* L. (var. AP3) seeds were acquired from the local seed distributor. Viable and uninfected seeds were picked and washed at first with 0.1% (v/v) sodium hypochlorite solution for 2– 5 min following thorough washing with Double Distilled water (DDW). Sterilized seeds were pre-soaked in different treatments of salinity sand salicylic acid for overnight as follows: T0 (distilled water); T1 (1 mM SA solution); T2 (50 mM NaCl solution); T3 (50 mM NaCl with 1 mM SA); T4 (100mM NaCl solution); T5 (100 mM NaCl with 1 mM SA); T6 (150 mM NaCl solution); and T7 (150 mM NaCl with 1 mM SA). Healthy seeds were then placed for germination in petriplates (15cm) having two layers of wet filter paper presoaked with DDW and kept in culture rooms at a light intensity of 100 µmol $m^{-2}s^{-1}$ and a 14/10 h (day/night) photoperiod for generation of complete plantlet. Irrigation was done twice in a week for different treatment plants with corresponding solution to keep the field capacity at 70–75%. The experimental condition was maintained through-out the study period. Analyses were carried out after 3 days, when obvious external differences were observed between the plants subjected to different treatments.

ANALYSIS OF GROWTH AND BIOMASS

After the completion of experiment (30 days), one intact plantlet (roots, shoot, leaf) were indiscriminately selected from each treatments. The plantlet height, fresh and dry weight of roots and stems was obtained by elec-

tronic scales. Total germination percent was calculated by using equation:

(TG) = (total number of germinated seeds/total seed) × 100.

RELATIVE WATER CONTENT

A 0.5 g of freshplant sample was weighed (fresh weight, WF) in the wake of rehydrating for 24 h oblivious (soaked saturated weight, WS) and after broiler drying at 85°C for 24 h to a steady weight (WD). The RWC was computed utilizing the equation:

RWC(%)=[(WF-WD)/(WS-WD)]

TOTAL FREE PROLINE CONTENT

Proline analysis was done by techniques portrayed by Bates et al (1973) with a few adjustments. The absorbance was measured at 520 nm utilizing toluene as a blank. The proline concentration was estimated utilizing proline standards (0-50 mg/mL).

Determinations of photosynthetic pigments (total chlorophyll and total caretenoids)

The determination of chlorophyll pigments was done by Arnon (1949) method with some modification. Absorbance were taken by uv-visible spectrophotometer at 645 nm for chlorophyll'a'and 663 nm for chlorophyll 'b' using 80% acetic acid as a blank. Calculation was done by using the following equations:

Chlorophyll a: 12.7(A663) – 2.69(A645)
Chlorophyll b: 22.9(A645) – 4.68(A663)

The Carotenoid estimation was done by Kirk and Allen, 1965 by using equations

Carotenoids = A480 + (0.114 × A663) – (0.638 × A 645)

TOTAL PROTEIN CONTENT

Total protein content was estimated in each sample through the method of Lowry et al, (1951). The proteins were quantified using the supernatants of samples with BSA as a standard.

Assessments of Antioxidant Activity

For enzyme extract, 0.3 g of plant sample was crushed in a chilled mortar with 8 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1% polyethylene pyrrole (PVP) at 4°C. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. Supernatant were used to quantify the activity. SOD activity was evaluated by monitoring its capability to obstruct the photochemical reduction of nitrobluetetrazolium salt (Giannopotitis and Ries, 1977). CAT activity was measured by degrading of H_2O_2 (Aebi (1984). The POD activity was estimated using guaiacol as standard (Klapheck et al., 1990).

DATA ANALYSIS

Data were tested using analysis of variance (ANOVA) with Graphpad Prism version 5, and graph was plotted on MS tools version (10) Duncan's multiple range tests was used to detect differences between means. The P-value was set at 0.05 and 0.01 for the ANOVA and Duncan's multiple range tests, respectively.

RESULTS AND DISCUSSION

Salinity treatments significantly decreased plant growth in terms of germination and plant height of *Pisum-sativum*. On comparison with the non-salt-treated *P. sativum* (T0), the salt treatment 50, 100,150 mM NaCl significantly reduced the germination percentage and plant height by 16.09, 39.08 49.4% and 42.85%, 58.92% 60.71% respectively (Fig-2A). The SA treatment abridged the decrease in the germination and plant height of the salt-stressed sample. It was observed that salt treatment significantly decreased the germination percentage and plant height in dose dependent manner. Moreover when compared with SA treated plants, significant increase 9.58% and 26.4% in germination percentage and 53.3%, 34.2% in plant height under 50, 100 mM NaCl treatment were reported. In plant sample treated with salt concentration 150 mM (T6), plant height significantly decreased by 22.3% ($P<0.05$). However, there was no substantial difference in treatment with and without SA under 150 mM NaCl ($P<0.05$, Fig-2B)

Salinity also showed pronounced reduction in the dry mass of the shoots and roots. Compared with non-salt treated *P. sativum* seedlings (T1), the 50, 100,150 mMNaCl salt treatment reduced the dry weight by19.04%, 38.09%, 61.09% in shoot and 26.6%, 40.1% and 66.6% in root (Table-1). The SA mitigated the adverse effect of salinity by improving growth of stressed plant.SA treatment significantly increased the dry mass of the shoots, roots, and (shoot+roots) by 15.3%,22.3% and 37.5%, under 100 mM NaCl conditions, and 37.5%, 60% and 45% under 150 mM NaCl conditions ($P<0.05$). There were no noticeable changes observed in the dry mass of shoots and dry mass of roots between SA treatedand non-treated plants under 0 mM NaCl (T0) and (T1) condition i.e. in unstressed samples.

The RWC is an important parameter in estimating the physiological status of plants. When compared to control (T0), the RWC decreased by 27.2% and 40% under 100 mM (T4) and 150 mM (T6) saline conditions respectively with a significant level ($P<0.05$). SA treated plant showed reduced salinity effect on the RWC reduction in

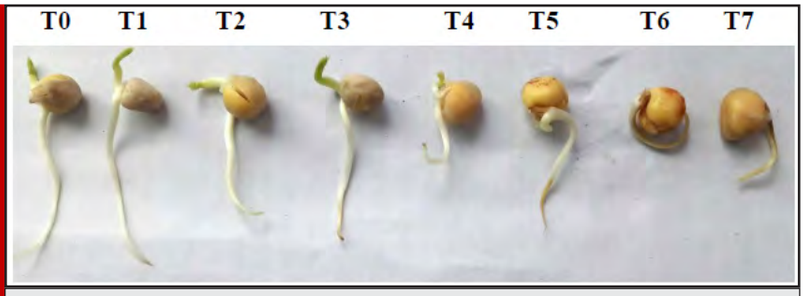

FIGURE 1. Effect of SA on seed germination of *Pisumsativum* under salinity stress.

the 0 mM and 50 mM NaCl saline condition; however, significance level was found (P>0.05). Under, 100 mM NaCl level of salinity,a significant improvement in RWC was recorded in plants treated with SA (P<0.05, Fig-3A). Salinity drastically prompted the deposition of free proline content in stressed plants. SA treatment significantly improved the proline content at all salinity levels (0, 50, 100 and 150 mM NaCl) by 4.8%, 24.4%, 12.7% and 11.7% respectively (P<0.05, Fig-3B).

Salt stress causeda decline in the photosynthetic pigments such as chlorophyll a, b, total chlorophyll and carotenoid contents on compared with control. The chl'a' content reduced by 36.3% under the 50 mM NaCl salinity level 54.5% under the 100 mM salt condition, and 72.7% under the 150 mM saline condition. Whereas SA ameliorated detrimental effect by reducing the decrease in chl'a' content by 18.1%, 36.3% and 63.6% under 50, 100, 150 mM NaCl salinity respectively, when compared to non-SA treated plant (P<0.05). More drastic reduction in chl'b' were found as salt concentration increased from 50, 100, 150 mM by 23.4%, 61.7%, 76.5% respectively (P<0.05). Under salt stress conditions, the SA treatment

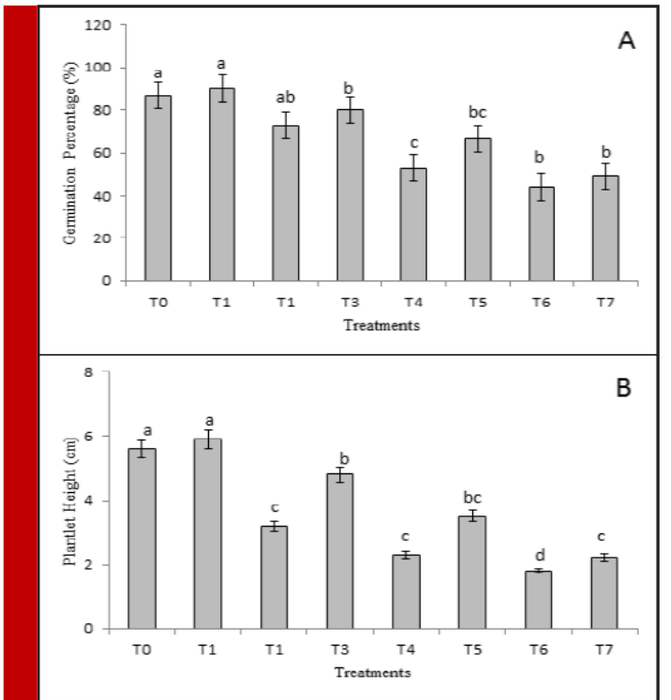

FIGURE 2. Effects of SA on the germination percentage (A) and plant height (B) in *Pisum sativum* under salt stress (means ± *SD*). Different letters indicate significant differences ($P < 0.05$); the same letter indicates no significant differences between the treatments, $n = 3$.

Table 1. Effects of SA on the dry matter of the shoots, roots, and roots + shoots in *P sativum* grown under salt stress (means ± SD). Different letters indicate significant differences (P < 0.05), the same letter indicates no significant differences between the treatments, n = 3.

Treatment	Shoot(g)	Root(g)	Shoot+ Root(g)
T0	2.1 ± 1.15[a]	1.5 ± 0.68[a]	3.6 ± 0.49[a]
T1	2.3 ± 0.93[a]	1.6 ± 0.39[a]	3.9 ± 0.98[a]
T2	1.7 ± 1.17[b]	1.1 ± 0.37[b]	2.8 ± 1.52[bc]
T3	1.9 ± 1.3[ab]	1.3 ± .074[ab]	3.2 ± 1.62[ab]
T4	1.3 ± 0.92[c]	0.9 ± 0.56[c]	2.2 ± 1.47[d]
T5	1.5 ± 1.34[b]	1.1 ± 0.45[b]	2.6 ± 1.10[d]
T6	0.8 ± 0.74[c]	0.5 ± 0.61[c]	1.3 ± 0.92[b]
T7	1.1± 0.89[a]	0.8 ± 0.56[b]	1.9 ± 0.76[c]

resumed the decrease in the chl'b' content by approximately 12.3% under the 50 mMNa Clcondition, 49.3% under 10 mM NaCl, and by 70.3% under the 150 mM NaClcondition. Similar responses were also observed in carotenoid and total chlorophyll content. Reduction percentage of carotenoid and total chlorophyll content under 100 mM and 150 mM was recorded by 66.6, 50.1% and 75.5, 72.2% respectively when compared to control (T0). From the above results, it could be concluded that SA significantly improved the photosynthetic efficiency by improving the pigment contents under different level of salinity (Table-2).

TOTAL PROTEIN AND SUGAR CONTENT

The protein content in *P. sativum* significantly (P<0.05) reduced in response to saline stress compared

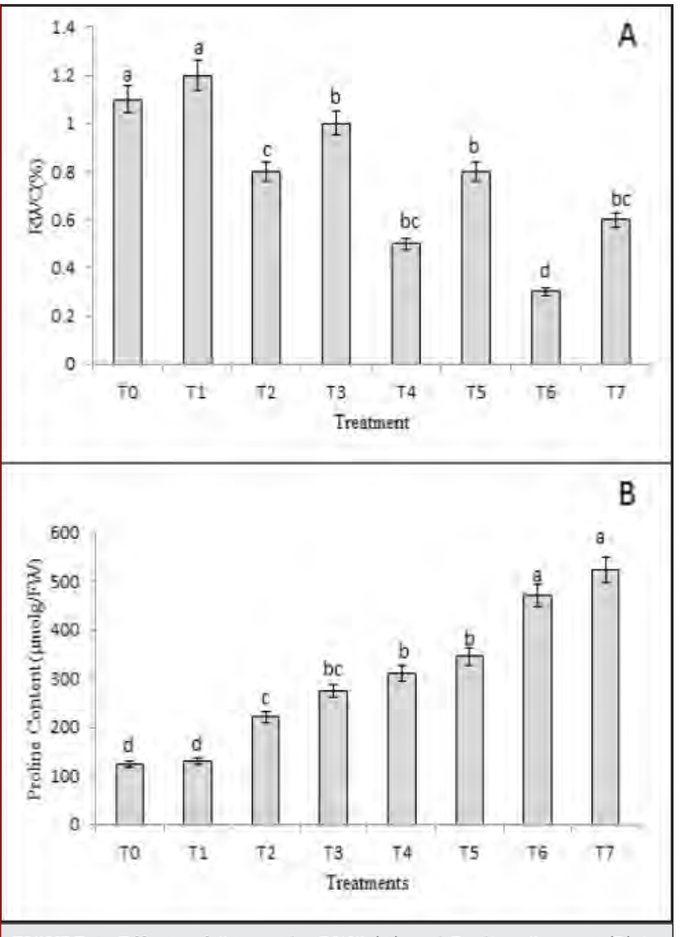

FIGURE 3. Effects of SA on the RWC (A) and Proline Content (B) in *Pisum sativum* under salt stress (means ± *SD*). Different letters indicate significant differences (*P* < 0.05); the same letter indicates no significant differences between the treatments, *n* = 3.

Table 2. Effects of SA on photosynthetic pigments in *P sativum* grown under salt stress (means ± SD). Different letters indicate significant differences (P < 0.05), the same letter indicates no significant differences between the treatments, n = 3.

Treatment	Chl a Content (mg/g)	Chl b Content (mg/g)	Car Content (mg/g)	Total Content (mg/g)
T0	1.1 ±0.74^{b}	0.81 ±0.41^{a}	4.2 ±0.14^{b}	1.8 ±0.035^{b}
T1	1.6 ±0.62^{a}	0.85 ±0.25^{a}	5.9 ± 0.11^{a}	2.4 ±0.014^{a}
T2	0.7 ±0.32^{c}	0.62 ±0.19^{c}	3.5 ± 0.05^{c}	1.2 ±0.002^{c}
T3	0.9 ±0.64^{bc}	0.71 ±0.23^{bc}	3.9 ± 0.15^{bc}	1.4 ±0.004^{bc}
T4	0.5 ±0.15^{de}	0.31 ±0.09^{d}	1.5 ± 0.25^{d}	0.9 ±0.006^{d}
T5	0.7 ±0.23^{d}	0.41 ±0.05^{d}	2.2 ± 0.19^{d}	0.7 ±0.009^{d}
T6	0.3 ±0.63^{e}	0.19 ±0.09^{d}	1.1 ± 0.48^{d}	0.5 ± 0.001^{d}
T7	0.4 ±0.59^{de}	0.24 ±0.08^{d}	1.4 ± 0.25^{d}	0.6 ±0.002^{d}

to their controls (T1). The content of protein decreased by 29.1% under 50 Mm NaCl, 56.4% under 100 mM NaCland 74.8% under 150 mM level of salinity. The presoaking treatment of SA significantly improved the protein content by 12.59%, 48.1% and 66.9% with in 50, 100 and 150mM salinity levels respectively (P<0.05, Table-3). Interestingly, sugar content showed dramatic increase by 58.1%, 130.2% and 312.2% on increasing salinity level from 50, 100 and 150 mM NaCl. Here also SA significantly improved the sugar

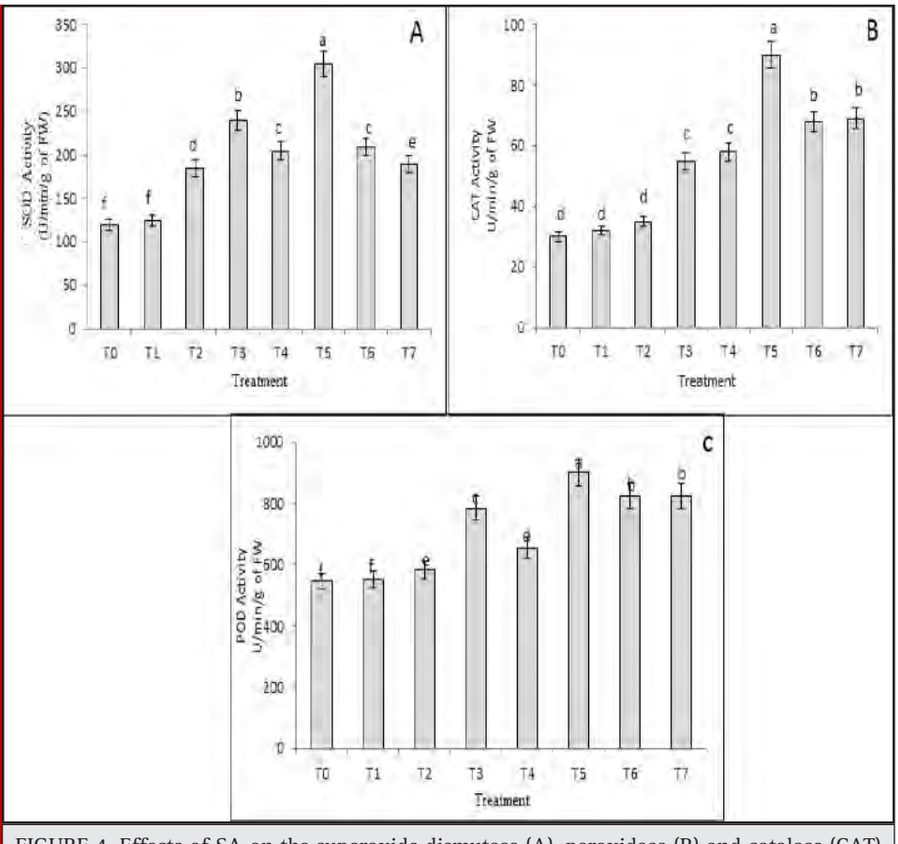

FIGURE 4. Effects of SA on the superoxide dismutase (A), peroxidase (B) and catalase (CAT) (C) in *Pisum sativum* under salt stress (means ± *SD*). Different letters indicate significant differences ($P < 0.05$); the same letter indicates no significant differences between the treatments, $n = 3$.

Table 3. Effects of SA on the Total protein and sugar content in *P sativum* grown under salt stress (means ± SD). Different letters indicate significant differences ($P < 0.05$), the same letter indicates no significant differences between the treatments, n = 3.

Treatment	Protein Content (μg g^{-1} FW)	Sugar Content (mg g^{-1} FW)
T0	67.56 ± 1.23^{b}	39.13 ± 1.05^{h}
T1	70.21 ± 1.06^{a}	47.02 ± 0.45^{g}
T2	47.80 ± 0.56^{c}	62.14 ±1.41^{f}
T3	59.01 ± 0.41^{bc}	76.08 ± 0.89^{e}
T4	29.40 ± 0.71^{d}	90.21 ±0.77^{d}
T5	35.60 ± 0.87^{cd}	120.09±1.23^{c}
T6	17.43 ± 1.65^{e}	161.41±0.89^{b}
T7	22.30 ± 1.67^{f}	185.25±0.98^{a}

content ($P<0.05$). But, no significant differences were observed between treated and non-treated plants in non-saline condition.

Quantification of Antioxidant enzymes SOD, CAT, POD activity

The antioxidant activities SOD, CAT, POD in the *P. sativum* were pointedly affected by salt and SA treatment (Fig-4). SOD increased by 36.3% 50.3% and 48.3% under the 50, 100mM and 150 mM NaCl conditions, respectively (Fig-4A). Though, CAT increased by 34.2% and 63.4% under the 100 mM and 150 mM NaCl conditions, respectively, but no significant difference was found between the non-salt condition (T1) and the 50 mM NaCl condition (T3). Further SA found to mitigate the adverse effect of salinity by significantly enhancing the antioxidant activities. SOD and CAT activity increased by 25.7%, 42.6% and 64.4%, 48.8% under respective 100 and 150 mM NaCl salinity when compared to non SA treated plant ($P<0.05$, Fig-4A and 4B). Similarly POD activity was also increased by 18.4% under 100 mM NaCl and 38.1% under 150 mM NaCl saline condition (Fig-4C). However, no significant variation were observed in the (SOD, POD, and CAT activity between the SA and non SA treated samples in control conditions (T1) ($P>0.05$).

Salt stress confines plant development and morphology by unfavorably affecting different parts of physiology and natural chemistry, for example, photosynthesis, superoxide ion homeostasis, antioxidant enzymes, osmolyte aggregation, and proline metabolism regulation (Misra and Saxena, 2009; Roussos et al., 2013). The present investigation clearly indicated that *Pisumsativum* is highly salt sensitive plant and presoaking treatment of SA mitigated the deleterious effect of salinity by improving various morphological, physiological and biochemical parameters such as increasing germination percentage, plantlet height, fresh and dry weight, activation of antioxidant activity and also photosynthetic process.

P. sativum under low salt stress (50 mM NaCl) exhibited no ostensible inadaptability due to the saline conditions. Additionally, to a certain degree, the exogenous SA responded the salt stress-prompted growth inhibition of *P. sativum* under 100 mM salinity; however no enhancement occurred under 150mM salinity. In current study, salt stress markedly reduced the drybiomass after treatment with 100 and 150mm NaCl subsequently 30 days of treatment (Table-1). The degree of severity in root was more as associated to shoot because root is the first organ that faces the salinity stress. Besides, expanding indication suggests that SA treatment fundamentally eases the harmful impacts of saltiness on plant development (Shakirova et al., 2003). Iqbal et al. (2006) demonstrated that SA enhanced the inimical impacts of salt stress on the development of wheat cultivars.It has likewise been accounted for that SA-treated maize plants had higher dried mass contrasted untreated plants that were additionally developed under salt stress (Gunes et al., 2007). The variety in assignment of biomass to various organs might be vital to the accomplishment of a seedling to adjusting to another condition (Tang et al., 2015).

In the present investigation, increased RWC and accumulation of free proline in *P. sativum* seedlings following 30 days of salt worry under SA treatment may be a versatile element in enhancing its succulence and keeping up the water adjust because of salinity actuated osmotic stress (Fig. 2A). These results are partially similar to the other study conducted on plant showed that proline accumulation regulate osmotic balance at the cellular level, retain membrane integrity and therefore, combat the injurypersuaded by salt (Misra and Saxena 2009; Ma et.al, 2017),.

The diminishment of leaf chlorophyll under high saltiness has been credited to the pulverization of pigments and the unsteadiness of the pigment protein complex (Jaleel et. al., 2009). The expansion of SA to NaCl-focused on plants particularly enhanced the photosynthetic content, proposing that the upsurge in chlorophyllpigments on treatment withSA may be due to capability of SA to improve the movement of specific proteins, consequently animating chlorophyll biosynthesis or decreasing chlorophyll debasement, prompting expanded overall photosynthesis process in salt stress resistance.

The decrease in chlorophyll because of osmotic stress attributed to the major harm to chloroplast layers, which builds the membrane penetrability or loss of membrane uprightness (Tang et. al 2015). Studies have demonstrated that the serious harm caused by saltiness stretch is in part because of the era of responsive oxygen species (ROS, for example, hydrogen peroxide (Asada, 2006). The upsurge in ROS was slowed down in plants

when presoaked with SA, proposing that SA possibly protect cells and sub-cellular systems from ROS cytotoxicity. In fact, plants can protect their tissues from the toxic effects of salt-accumulated ROS by using enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Verhagen et al., 2004). SA found to persuade the activities of antioxidant enzymes in the *P sativum* under the salinity condition (Fig-3). Otherstudy alsostrongly support that the SA enhanced antioxidants-activity, which protect the plants from oxidative damage (e.g., Hayat et al., 2008). In recent times, studies have showed that SA also helps incontrolling antioxidant enzyme activities to withstand salinity-induced injury (Horvath et al., 2007; Harfouche et al., 2008).

CONCLUSION

From the results obtained in the present study, it could be concluded that *P. sativum* is a salt-sensitive leguminous crop and severely affected by salt stress that's lead to the accumulation of ROS, debasement of photosynthetic pigments which resulted in reduced photosynthesis,growth inhibition and reduced biomass production.. However, SA curtailed the lethal effect of salt on the growth and adaptation of plant to saline environment, which was accredited to high activity of the antioxidant enzymes. However further extensive research required to elucidate the mitigating mechanism of SA in stressed plants.

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Evaluation of satisfaction rate of dental implants in patients referred to private clinics of Kermanshah city

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ABSTRACT

The satisfaction of dental care has always been an important topic in the field of dentistry. Several studies in the country from different parts of the dental patient satisfaction has been made. But few specific studies on the satisfaction of patients of the dental implant have been done. This study was aimed to assess the satisfaction of implantation done in the city of Kermanshah. Since the present study, is a retrospective study, 108 cases of dental implants placed in selected patients were asked to fill a questionnaire that included demographic information and questions related to the satisfaction of dental implants placed were asked. Data were collected and analyzed using software SPSS18 were analyzed. The results showed that the level of patient satisfaction in the satisfaction of the cost, 80%above the average and 9.7 is weak. Patients' satisfaction with the performance of, and only 2.7 percent of the poor were good average 3/97. The findings also showed that mental satisfaction and overall satisfaction of patients was 100%.But the satisfaction of performance including waiting time was less than the satisfaction rate of 51 percent good and 49 percent were poor.

KEY WORDS: FELDSPATHIC PORCELAIN, IPS E.MAX HEAT PRESS LAYERED, ENAMEL TOOTH WEAR

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INTRODUCTION

Despite the many advantages that conventional prosthetics have in restoring the beauty and oral function of patients, their deficiencies cause dissatisfaction of patients in many cases, and this leads patients to tend to use more durable and more satisfying treatments, one of which is dental implants. The lack of serious defects and the problems of moving and fixed prosthesis, such as damage to adjacent teeth, Ridge jawbone fracture, undesirable beauty, and other defects in conventional prosthesis by replacement of bone implants have increased their usage worldwide, so that the use of dental implants in the United States has grown ten times during 2002-1983 years (Annear et al 2005) and (Karoussis et al 2003) and (Behneke 2002). With the advent of the osteointegrationtheory in dentistry and proving that pure titanium in direct contact with the bone could provide a good support for replacing missing teeth, then it led to the introduction of dental implants (Wheeler 2003).

At first, dental implants were used to maintain the function of the patients, but later it was added to the beauty. Along with the supply of various implant systems, many clinical studies have been conducted to assess their durability rates. Edleau et al. (1981), in their 15-year review, reported success rates of 84% in upper jaw and 93% in mandibles (Adell et al 1981). High success and benefits such as the lack of need for adjoining tooth cuttin and the prevention of alveolar bone fraction have led to an increase in the use of dental implants (Hobo 1997) and (Lekholm 1999) and (Lindquist et al 1997).

Following the protocol provided by Branemark is often recommended to succeed in achieving successful osonetic secretion (De Bruyn et al 1995). Saha et al. (2013) in a study questioned 403 people to assess the awareness rate of dental implants whilethe results showed that half of them had completely no information about the implant (Saha et al 2013). These studies are consistent with recent studies of the low level of data about dental implants (Awad et al 2013). The lack of accurate and true information is one of the reasons that increase the unrealistic expectations of patients, which reduces their satisfaction (Satpathy et al 2011). The aim of this study was to evaluate the satisfaction of dental implants in patients treated with dental implants.

MATERIAL AND METHODS

In this analytical-descriptive study, in order to evaluate the satisfaction of implantation, 108 archival records of patients treated with dental implants were randomly selected using a randomized table referring to dental clinics in Kermanshah. In order to collect the research data, the researcher completed a questionnaire including demographic data and researcher made questions to assess the satisfaction of dental implant implantation by calling the patients and inviting them to face-to-face interviews. The scores of the questionnaire were ranked based on three good/medium/bad grades, which 1-2 as weak, 3-6 as average and 7 to 10 as good were ranked. Also, 1-2questions evaluate cost satisfaction; 3-6 questions evaluate satisfaction from implant function, and questions 7-10 assess mental satisfaction and satisfaction with the stages of work. Mental satisfaction is one of the modes of satisfaction (Levi et al 2003), which is calculated based on the score that a person received from the mental satisfaction questionnaire of the research questionnaire from questions 7 to 9.

The study population was selected from patients treated with dental implants who had undergone at least one year of treatment. The sample size was calculated based on a pilot study and considering the variables of satisfaction such that S=3.17 (standard deviation), $\alpha = 0.05$ (confidence), and d = 0.6 (accuracy), while the minimum sample size was 108, which was selected as convenience.

$$n = \frac{Z^2_{1-\frac{\alpha}{2}} S^2}{d^2} = 107.229$$

The collected data was analyzed by SPSS software version 18. In descriptive statistics, mean and frequency distribution and in inferential statistics, Mann-Whitney test were used.

RESULTS

The level of the cost satisfaction of the people was assessed9.7% as weak, 47.8% moderate, and 42.5% as good.The satisfaction of individuals with implant function was evaluated as 2.7% weak, 4.4% moderate and 92.9% good. The mental satisfaction of individuals was evaluated as 0% weak, 16.8% moderate and 83.2% good. The degree of satisfaction of the people from the stages of work was evaluated as 48.7% weak and 51.3% good (Table 4-6 and Fig. 4-8). The satisfaction of beauty was evaluated 15% were weak and 85% good. The satisfaction rate of the whole population was evaluated 0% weak, 10.6% moderate and 89.4% good. The total satisfaction of the individuals by gender was evaluated in men, 0% weak, 10.6% moderate and 89.4% % good and in women 0% weak, 10.8% moderate and 89.1% good.

In order to determine the relationship between satisfaction and age variables, implant loading years, gender and education level, Chi-square and Fisher's exact tests were used. Chi-square test showed no significant

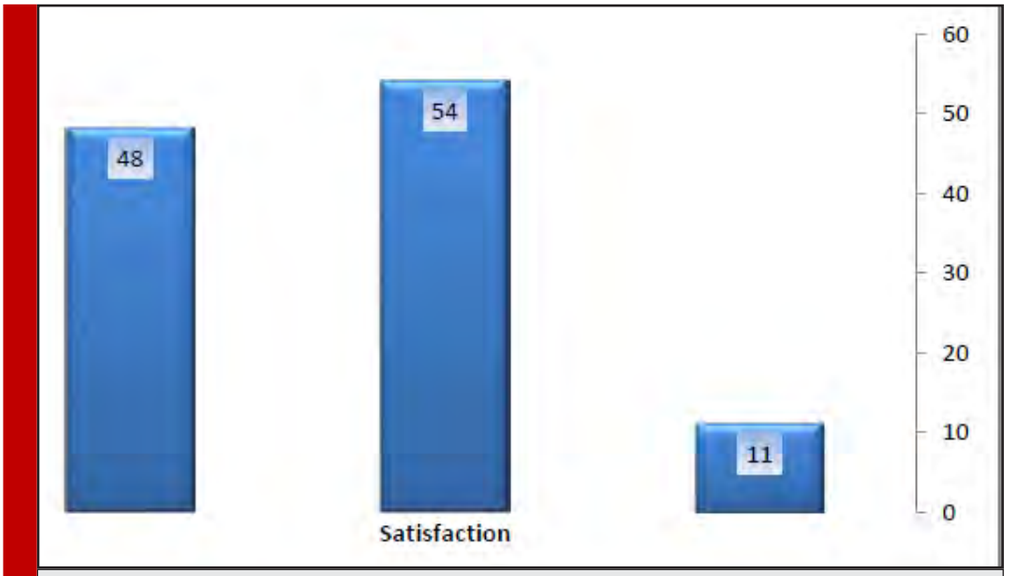

FIGURE 1. Distribution of Cost Satisfaction among Participants in the Study

relationship between satisfaction and age (forty-five years or less - more than forty-five years) (P-value = 33.93). Fisher's exact test showed no significant relationship between satisfaction and implant loading years (three years or less, more than three years) (P-value = 513). Chi-square test showed no significant relationship between satisfaction and gender (P-value = -0.96). Fisher's exact test showed that there was no statistically significant relationship between satisfaction level and level of education (diploma and undergraduate - university degree) (P-value = 0.96).

DISCUSSION AND CONCLUSION

Research findings in the area of satisfaction from the cost of implanting showed that almost ninety percent of

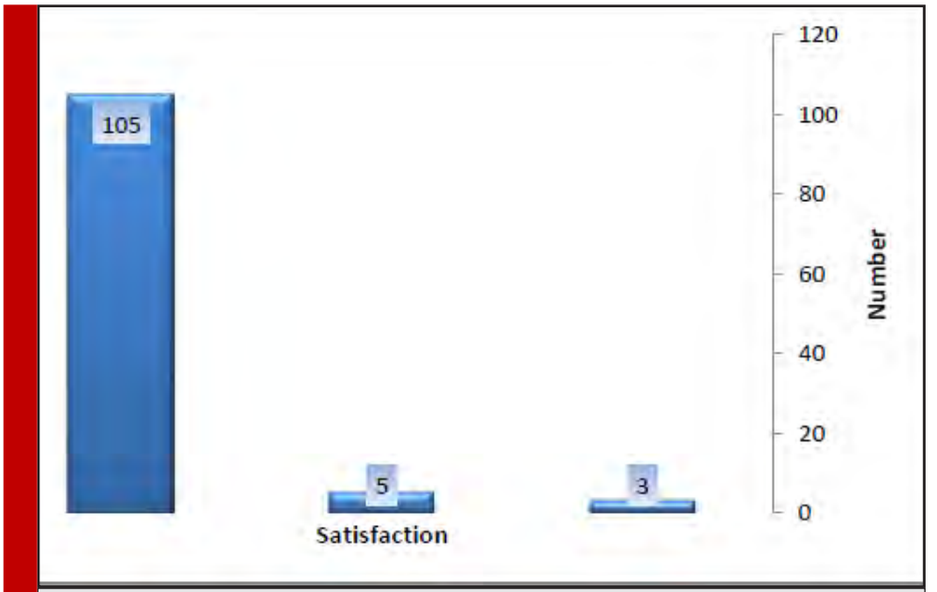

FIGURE 2. A Distribution of satisfaction with implant function among participants in the study

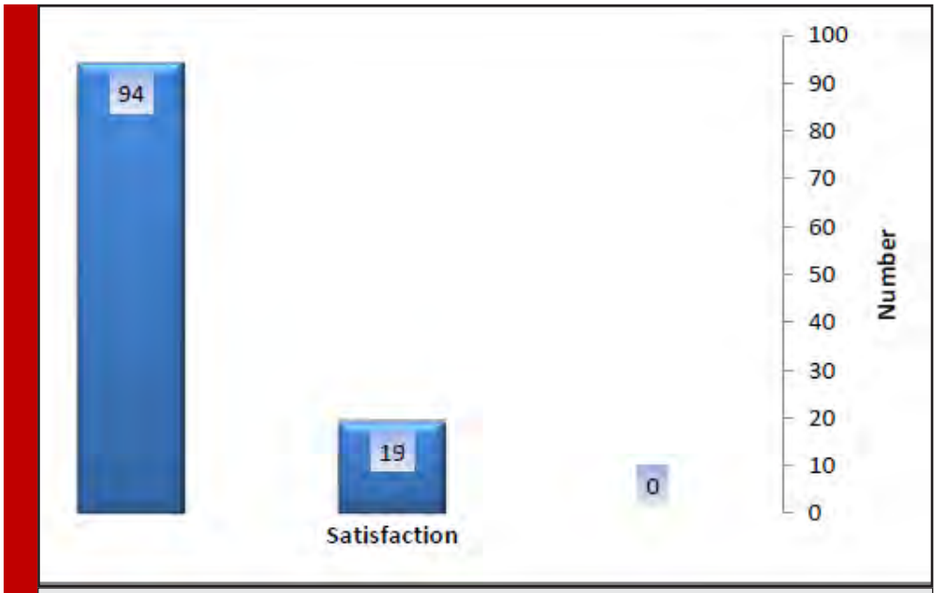

FIGURE 3. Distribution of mental satisfaction of participants in the study

participants were satisfied with the cost of implanting. It is clear to everyone that the dental services in our country are expensive due to the cost of dental materials and its costs. Patients always expect the services to be satisfactory and cost-effective. The results of this study showed that the patients in this study were satisfied with the cost of implant implantation, which indicates that patients' expectations have been met. This means that although the inserted implant imposed cost on them, but it has been able to keep them satisfied with this action. The results of this study were consistent with the results of study by Rismanchian et al. (2007). The Rismanchian et al, in their study reported a satisfaction of over 90%. In their study, 107 male and female patients were evaluated. Their study was a two-year study, but the current study was a one-year study after implant implantation,

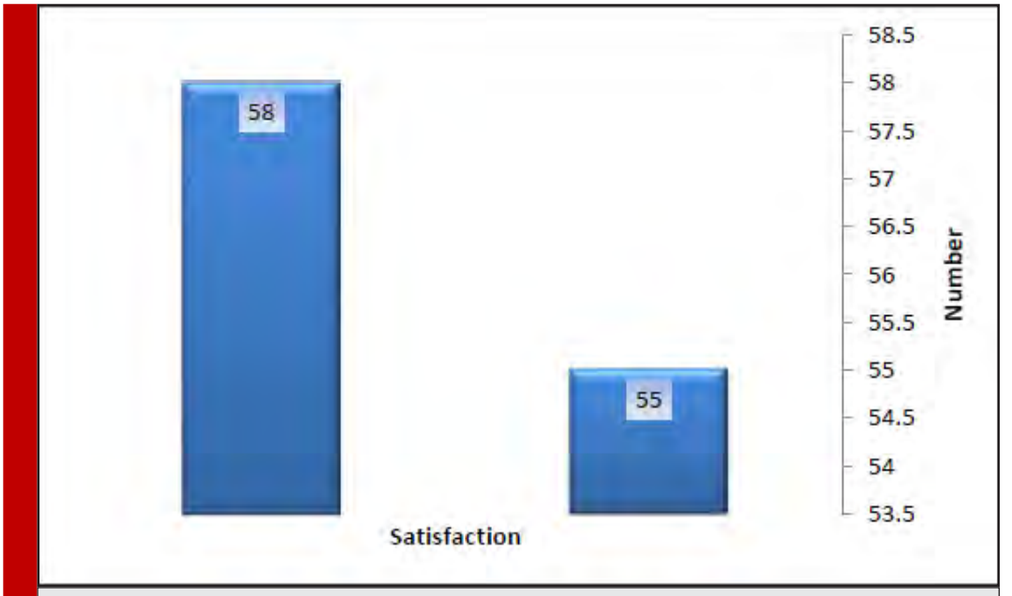

FIGURE 4. Distribution of satisfaction from work stages among participants in the study

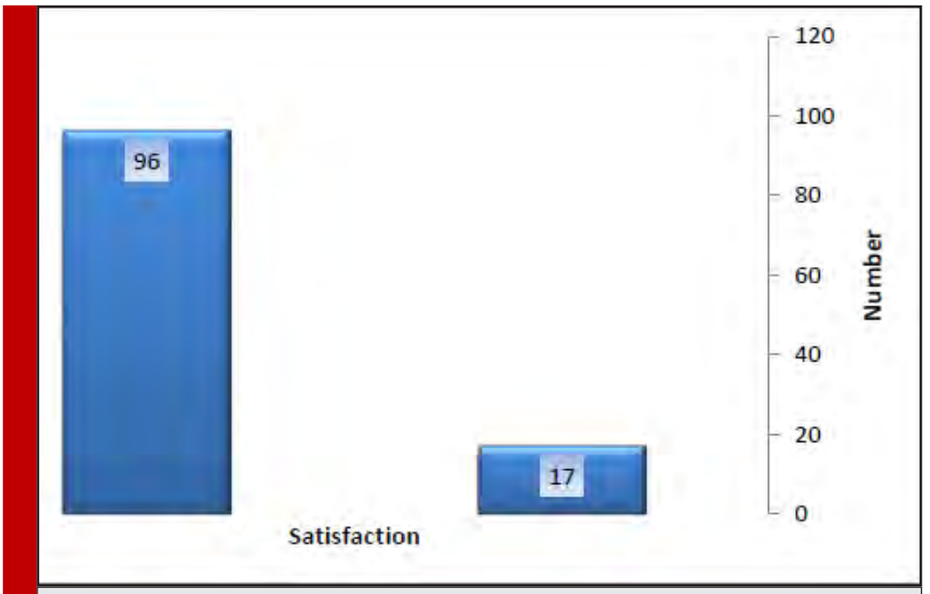

FIGURE 5. Distribution of satisfaction with beauty among participants in the study

however, high satisfaction was reported in both of the mentioned studies.

The findings of the study showed that all individuals are satisfied with implanted implants. More than 92% of the patients in the study showed a moderate to high satisfaction with implant function. Implant function is very important for the patient. The results of this study are consistent with the results of studies by Pjetursson et al (2005), Levi et al. (2003). They reported in their studythat more than 90% of patients who have implants are satisfied with implant function. In studies ofPjetursson et al. (2005) implanting time was for 5 to 15 years. But the current study was a one-year study, but the results in both studies showed a high satisfaction of patients from implantation.

It is worth noting that Pjetursson et al. (2005) used VAS for measuring patients' satisfaction in their study, but in the current study, only a questionnaire was used

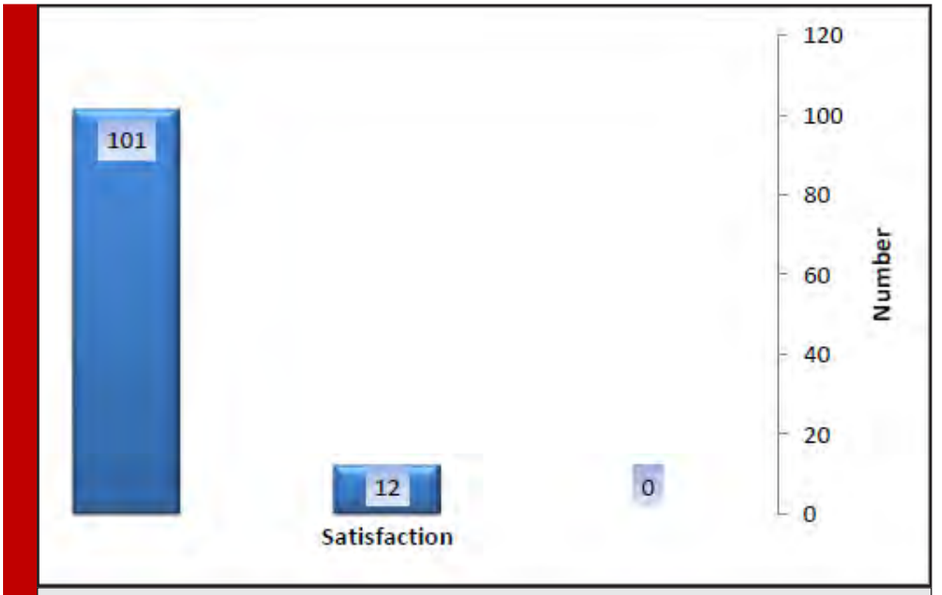

FIGURE 6. Distribution of satisfaction among participants in the study

Pjetursson 2005). The primary purpose of the implant is to help the patient's function, and this goal is still important for patients. An implant satisfaction survey shows that satisfaction with implants is high in patients and it has been able to meet patient expectations (Jie Yao et al 2014). In their study, patients were satisfied with the condition of the implant, shape, overall appearance, effect on the speech, and the ability of the function. The results of the current study also confirm this satisfaction. Although satisfaction was used in their study using email, the degree of satisfaction in the current study was consistent with their study.

The findings of the research in the area of mental satisfaction showed that more than 80% of the patients had a high satisfaction with the implants, while the findings showed that only 16% of the patients had low mental dissatisfaction. Mental satisfaction is a condition in which a person is satisfied with the work done and has internal satisfaction (Levi et al2003), which is calculated, based on the score obtained from the mental satisfaction questionnaire of the research questionnaire, which are these questions 7-9. The results of this study were consistent with the results of Levi et al. (2003).

They reported in their study that patients who have dental implants had a high degree of mental satisfaction with their implants. Patient's mental satisfaction can reveal a set of person's satisfaction with cost, function, beauty. Patient mental satisfaction can be considered as the most important type of satisfaction, because if there is no mental satisfaction, it can be claimed that the work has failed. But given the fact that some patients have very high expectations from implants, their implant satisfaction is not provided after implantation. This can be argued that a dentist must reach the patient's expectations before implantation, and moderate the unreasonable mental expectations of the patient (Levi et al 2003). Patients are expecting miraculous treatments by referring to dental centers and hearing some of the new techniques. Dentists can make the patient's mental expectations largely acceptable with a simple explanation of the stages of work and problems that may occur during work.

The findings of the study showed that only half of the participants were satisfied with the stages of work, and the other half were completely dissatisfied with the work process. Dissatisfaction with the work process involved the waiting time until the layout of the cover. Dental services, and especially implants are usually time-consuming due to the collaboration of several specialists and labs, but the patient expects to achieve the goal at a very short time. It is necessary to the dentist explain the required steps to increase the patient's satisfaction, the need for collaboration of several specialists and the laboratory, and the time of implantation for the patient prior to the implant implantation (Razmi et al 2010), which would increase the patient's satisfaction. Dental services are always time-consuming due to the special circumstances. Implanting will naturally be more time consuming due to the collaboration of several specialists and lab. This is necessary to tell the patient at the beginning of treatment. Explaining the patient in addition to patients about the collaboration reduces their expectations, can also increase patient satisfaction from work.

Also, the findings showed that approximately 90% of total satisfaction in patients was well evaluated, and only 10% of the participants had a moderate overall satisfaction rate. The results of this study were consistent with the results of studies by Pjetursson et al. (2005), Levi et al. (2003), Yi et al. (2001) and Riesmannchian et al. (2007). In their studies, they reported total satisfaction in patients who had implants, including satisfaction with function, beauty, cost, and acceptable levels (Razmi et al 2010) and (Yi et al 2001) and (Rismanchian et al 2007) and (Haji et al 2010). The findings also reported the equal overall satisfaction in male patients and female patients, and did not show any difference between the satisfaction of women and men. It can be said that the most important issue at the end of dental care is the patient's satisfaction. Although the work process and services provided by the dentist may be confirmed, it is more important than the patient's own satisfaction, and it should be noted that satisfaction is a mental issue. In the current study, the satisfaction of implant implants was reported, which indicates that dentists have been able to achieve a satisfactory level of satisfaction at all stages. In all studies, overall satisfaction has always been reported with high rates. This may lead to an increase in implant implants in Iran and other countries in recent years.

CONCLUSION

According to the mentioned materials, it can be said that dental services in the field of implanting have been able to achieve satisfactory patient satisfaction. Implanting has been able to satisfy patients in the issue of satisfaction with performance, cost, and satisfaction. Only on the work process the patients were dissatisfied while it should be noted that the dentist needed to justify the patient from the beginning of the implant implantation timeliness, the steps required, the need for several specialists and the laboratory to work together, which could moderate the patient's expectations. This will increase patient satisfaction. It should be noted that satisfaction is a mental issue and dentists have to meet the expectations of the patient, especially patients with unreasonable expectations and many expectations of implant implantation treatments, at the beginning of the treatment so that they can increase the level of satisfaction of the patient.

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Psychological Communication

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Investigations on the relationship between perfectionism and psychological needs with the perception of body image in cosmetic surgery applicants

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ABSTRACT

Physical appearance is an important part of the body image because it is the first source of information which others use it for social interactions with that individual. Every year tens of thousands are doing cosmetic surgery in order to eliminate dissatisfaction with their appearance and sometimes to increase self-esteem. The aim of this research was to investigate the relationship between perfectionism and psychological needs with the perception of body image in cosmetic (plastic) surgery applicants. Research population consists of all the men and women who were applicants for cosmetic surgery and had recourse to beauty clinic of Imam Khomeini hospital, Tehran, Iran. 200 individual samples were selected by availability sampling method. The method of this paper was descriptive-analytical and data collection is done using questionnaire. data were analyzed using SPSS. Results showed that there was a relationship between perfectionism and psychological needs with the perception of body image of cosmetic surgery applicants. Psychological and perfectionism needs and also the perception of body image of women are different from men's. In addition, the negative characteristics of perfectionism have a larger share in cosmetic surgery applicants' prediction of body image perception.

KEY WORDS: PSYCHOLOGICAL NEEDS, PERFECTIONISM, THE PERCEPTION OF BODY IMAGE

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INTRODUCTION

Physical appearance is an important part of the body image because it is the first source of information which others use it for social interactions with that individual. There is no official statistics on cosmetic surgery in Iran. According to unofficial statistics, every year, more than 36 thousands of Iranians do facial cosmetic surgery. Among the performed cosmetic surgeries in Iran, rhinoplasty is in the first place. According to unofficial statistics, from the numerical aspect of rhinoplasty, Iran is the first in the world. After Iran, countries such as America, England, and partly France are in the next ranks. Approximate statistics were only unofficial sources of cosmetic surgery. Those people, who desire to do these types of surgery but fear, should be added to this statistics. The concept of an individual's perception of their body is an important part of their self-concept. The body is the most visible part of ego and consciousness. Body image is person's internal visualization of external appearance which includes representation of physical, cognitive and attitudinal dimensions. The main dimensions of this attitude consist of components like evaluation and individual investment in dealing with schemes of appearance and emotion which reveal the importance of internalized appearance, (Sarwer, 2003, Cash, 2004, Borzekowski, 2005, Balali, 2010, Weiss, 2013, Nerini, 2014).

In the twentieth century, especially in recent decades, new concepts of beauty have emerged and beauty is defined as a collection of components such as fitness, makeup, clothes, attractiveness, and integrity. The combination of beauty and industrial and also medical achievements has caused that now, beauty is not a natural and biological characteristic anymore and it is an adventitious trait. Nowadays, cosmetic surgery is as one of the most common surgical procedure on a global level that the use of which is growing. The main purpose of cosmetic surgery is improving the appearance of the person who has the abnormal appearance. When people find out their physical properties are not in accordance with those norms, may be offended and consider the surgery as the final solution. Now, cosmetic surgery becomes an agent charming and luxury-oriented means to different people, and this has led to the establishment of people's unrealistic expectations and distortion of the facts about its true nature, (Hilhorst, 2002, Pearl, 2003, Hwang, 2004 Wolf, 2013).

These days, cosmetic surgery is taken into consideration for creating beauty and desired image, so the image of beauty as God's natural creation is drawn to adventitious and clinical beauty. With the increasing demand for plastic surgery, several studies of this field have introduced some effective factors on the desire to these types of surgery. These factors are dissatisfaction with body image, low attractiveness, psychological factors, body dysmorphic disorder, precedent of previous cosmetic surgery, social and interpersonal factors such as annoying appearance from the perspective of others, surgical precedent of family and friends, cultural factors, the impact of mass media advertising on TV, satellite, magazines, movie stars, stress pressure, medical System and beauty industries. At the end of the twentieth century, a major concern about body image was clearly felt, (Garrusi, 2013).

This concern has mainly surrounded women. Women whose body image has much importance for them, discontent and dissatisfaction of appearance is associated with their dissatisfaction of themselves and in general, with their life. Orientation about beauty is in all social situations. There is an irrational but firm belief that attractive people possess the other ideal characteristics such as intelligence, adequacy, social skills, confidence and even much moral values. Of course not surprising that physical attractiveness is very important; however, this as well as concerns about physical appearance is not limited to this century and at any time and historical period has been important and some criteria are determined for beauty and ugliness. Efforts of individuals across the life is attempting to what look like to show themselves in the best possible way. Physical appearance affect on others, even if it be accepted that these effects can be changed after the formation of communication (Rumsey, 2004).

Benfield and McCabe declared that body image has three dimensions: perceptual-evaluation, emotional and behavioral dimension. Perceptual-evaluation dimension refers to individuals' measurement and evaluation of their body. Feelings and emotions which individual experiences from her body lies in emotional dimension; and behavioral dimension focuses on the effect of persons' thoughts and emotions about their body on their behavior (Banfield, 2002).

Body image is consisting of two aspects: perceptual aspect (individual's evaluation of her body sizes) and emotional-cognitive aspect (person's attitude toward her limbs). According to available findings, the assumption is that when persons know their body lower than ideal criteria, may encounter with inappropriate feelings and attitudes about themselves like low self-esteem or depression (Pesa, 2000) and in some cases even causes a dropout. Cash introduced evaluation, emotion, and investment as three main dimensions of body image dimensions (Cash, 2004).

Socio-cultural theory as a prominent theory in the field of body image, and by raising the social and cultural variables on body image disorders, has received much empirical and research support. Of course, most

of the studies have focused on women's social and psychological variables. This focus probably is due to the high prevalence of body dissatisfaction and body image disorders in women and young girls. The investigation found that the incidence of physical ideal for women is associated with their increasing body dissatisfaction (Hargreaves, 2006). Studies show that awareness and internalization of women's ideal are predictors of body dissatisfaction, body image, and a decrease of self-esteem. Cosmetic surgery includes carrying out all cosmetic surgery, including nose, eyelids, eyebrows, cheeks, forehead, neck and body beauty which may change (such as removing fat) or improve (e.g. dentures). Generally, cosmetic surgery refers to surgeries without the special medical necessity that is done solely for cosmetic purposes (Tavassoli, 2012).

Surgical breast implants in the United States is eight times since 1992. Also in 2001, 1.6 million people have been surgery Botox injections therein. This rise of statistics is also seen among the male population. In 2002, the number of men who use Botox injection for less showing their age has increased 88% and men who have undergone rhinoplasty surgery has increased 47% (Ibid). For years, the negative image of the body has known as one of the reasons for resorting to plastic surgery. But few types of research have studied body image after surgery longitudinally. Sarwar and his colleagues in 2002 evaluated body image of patients before and after of cosmetic surgery (Heyes, 2016). A significant decrease of patients' dissatisfaction of operated limb than before the cosmetic surgery was reported. They also suffer less embarrassment in front of others' attention to that specific limb or talking about it. But there was no change in studied women's general body image. These results represented that people who undergo cosmetic surgery are rarely significant changes in their general discontent. Therefore, cosmetic surgery is a therapeutic plan for people who are dissatisfied with just one part of their body and face (Sarwer, 2003).

Many factors can contribute to the negative image of the body, including physiological factors (such as body mass index), psychological factors (like perfectionism, low self-esteem) and socio-cultural factors (such as family, peers, and media). In the contexts of perfectionism, both one-dimensional and multi-dimensional approach to perfectionism can be seen (McGee, 2005). Person-centered divisions of perfectionism are normal – neurotic, satisfying- dissatisfying, and negative or positive perfectionism (Stoeber, 2007; Besharat, 2007). In the framework of person-centered approach, in addition to neurotic, abnormal and damaging roles of perfectionism which are more known, its normal and desirable and also positive effects are confirmed and clarified (Slade, 1998). According to this division, a normal perfectionist enjoys effort and competition for superiority and perfection and at the same time accept personal limits, although neurotic perfectionist due to unrealistic expectations, do not be satisfied with her performance (McGee, 2005).

Positive perfectionism: positive perfectionism (strive for positive progress) is to Try to achieve high personal standards without self-criticism. This aspect is not related to negative characteristics such as anxiety; positive perfectionism mainly focuses on expectations regarding progress, competition, capability, the excitement of a work and satisfaction. On the other hand, positive perfectionism is achieved by attaining positive and motivation to access a certain goal so that it has promising results (Haas, 2008). Despite the negative perfectionists (neuroticism), positive perfectionists (normal) at the same time that impose high personal standards for themselves, they can accept personal and environmental limitations and know their success, even if they do not meet their personal standards exactly. This group of perfectionists in an effort to succeed and achieving the goals, in terms of the ability to accept personal limitations and environmental barriers, can experience a sense of satisfaction more (Besharat, 2007).

Negative perfectionism: negative perfectionism dimension contains that form of perfectionism which explains perfectionism concerns such as too much attention to mistakes, doubts about negative actions and reactions. This dimension is associated with anxiety (Stoeber, 2007). Negative perfectionism is focused on emotions like not meeting expectations of the individual about herself and others, condemning, feeling guilty, shame and defeating (Haas, 2008). In addition, these persons strictly avoid failure and are vulnerable to criticism of others. This group of perfectionists treats the various spheres of social activity and relations as threatening, procrustean and not supportive (Besharat, 2007). The major difference between abnormal and dysfunctional perfectionism and positive and normal perfectionism is that in the first group despite conflicting evidence, perfectionist tendencies are continued (Figure 1).

The concept of basic psychological needs is completely different with the theory of personal efforts and wishes. Although people know the motivations as a provision of basic needs, but it is clear that there are many incentives that do not comply with the standards of welfare; in fact, it may be harmful. In the other words, may some incentives mislead the people about what can resolve their requirements and it means misleading their deviation of welfare? Even when people can satisfy their motivations, again the motivation can be harmful to the welfare if it barricades satisfying the needs of autonomy and communication (Ryan, 2000; Sheldon, 2001). Unfortunately, from the past till now there is little consensus about the criteria of psychological needs, where they

FIGURE 1. Perfectionism features

originated, whether thy are result of learning in childhood or cultural differences, whether they are inherent and universal and have been established by evolution in man; These are questions that need to be asked about (Sheldon, 2001). In this part, the importance of three psychological needs namely autonomy, competence, and relatedness are investigated. When people find themselves in an environment that helps them to develop their psychological needs and also fosters them, positive emotions, optimal experience, and healthy growth are its consequences. Psychological needs are important for the study of motivated behavior. The energy of psychological needs is pioneering. Psychological requirements induce a desire to seek the environment that is expected to bring up our needs (Azin, 2014).

Autonomy (independency): independency means person's need to electoral right and self-initiate for duties. For example, when people from the satisfaction and enthusiasm, spend their time and energy to study in order to feel autonomy (Ryan, 2000). Need to autonomy is a need to self-following and having the electoral right at the beginning maintenance and tuning the activities. Autonomy will happen when people feel the responsibility for their behavior, and confident in their choices and also the ability of eligible performance, (Sheldon, 2001). Autonomy means feeling volition, having authority of doing things, innate tendency for self-organized behavioral experience (Sheldon, 2001). Autonomy is related to freedom and experience of solidarity in personal behaviors.

Competence (adequacy): all people try and want to be competent. All want to have fruitful interaction with their environment and this interest is expanded to all aspects of life such as school, workplace, relations, and at the time of recreation and sport. All like to develop some skills and enhance abilities and talents. When a challenge is faced, all attention is focused on it. When the opportunities to develop the skills and talents is given all would like to progress. In these circumstances, the result is a sense of satisfaction. In the other word, human needs to competence; need to competence means the ability of a person in doing duties and that person have how much ability to achieve the desired objectives (Mulder, 2007).

Relatedness (continuity): relatedness is a need to emotional bonding with others and this need also expresses the desire of being emotional and being involved in intimate relationships. The requirement to relatedness causes to leading to people who certainly care about the well-being of a person and eschewing of people who do not care about her welfare. Need to relatedness is defined as ensuring communication with others and need to make self-experience as an individual worthy of love and respect. In the other words, individual requires to have a relationship with others and be supported by them (Johnston, 2010).

MATERIALS AND METHODS

The method of this paper is descriptive–analytical and data collection is done using questionnaire. For this purpose, 200 men and women who were an applicant of cosmetic surgery and had come to beauty clinic of Imam Khomeini hospital in Tehran and were chosen using availability sampling method. And also analyzing data is done using the SPSS19 software.

RESULTS AND DISCUSSION

In order to a comprehensive analysis of data, a statistical method of multiple regressions was used and data was investigated based on findings and hypotheses. First hypothesis: psychological and perfectionism needs predict the cosmetic surgery applicants' perception of body image. For determining the relationship between psychological and perfectionism needs and cosmetic surgery applicants' perception of body image, multivariate regression is used whose digest is reported in Table 1.

Table 1. Summary of multiple regression analysis

Model	R	R^2	Justified R^2R^2	Standard error
1	0.54	0.29	0.27	26.14

Predictor variables: psychological and perfectionism needs Criterion variable: perception of body image. As can be seen in the table, the intensity of the relationship between psychological needs (autonomy, competence, and dependence) and perfectionism (positive and negative) and cosmetic surgery applicants' perception of the body was calculated. Its value obtained 0.54 and they explain 20% of changes in cosmetic surgery applicants' perception of body image.

As it is shown in Table 2, in the regression model, psychological needs (autonomy, competence, and dependence) and perfection (positive and negative) has a significant relationship ($F=14.51$, $P<0.01$) with cosmetic surgery applicants' perception of body.The criterion variable: perception of body image.Results of the Table 3 indicate the share of each variable in the prediction of perception of body image model. As it is observable, positive and negative perfectionism factors have a significant share in the prediction of "body image perception" and the largest coefficients of beta equal to -0.37 belongs to negative perfectionism. In the other words, "negative perfectionism" factor has a stronger role in the prediction of "body image perception" in cosmetic surgery applicants.

Second hypothesis: psychological needs, perfectionism, and perception of body image of women and men who are an applicant of cosmetic surgery are different. At first, the assumption of equality of variances for psychological needs, perfectionism, and perception of body image was examined. As it is shown in Table 4, there is an equal assumption for psychological needs, perfectionism, and perception of the body of men and women applicants of cosmetic surgery.

After determining variances equality assumption, for comparing scores of psychological needs, perfectionism, and perception of body image of women samples, student's t-test was used for groups apart which results are reported in the table. According to Table 5, because significance level of "t" value (which is calculated by comparing psychological needs, perfectionism, and perception of image body of samples) is more than 0.05, the fourth hypothesis was not confirmed. Therefore, it can

Table 2. Summary results of analysis of variance for significance of regression model

Source of changes	Sum of squares	Degrees of freedom	Mean square	F	Significance level
Regression	49602.9	5	9920.58	14.51	0.001
remaining	116898.72	171	683.61		
Total	166501.62	176			

Table 3. Summary of regression coefficients for psychological and perfectionism needs

Final model	Regression coefficient (B)	Standard error	Standard coefficient (Beta)	t	Significance level
Autonomy	0.13	0.36	0.03	1.35	0.72
Competence	0.48	0.40	0.10	1.20	0.23
Dependence	0.56	0.31	0.13	1.81	0.07
Positive perfectionism	1.21	0.36	0.24	3.34	0.001
Negative perfectionism	-0.68	0.13	-0.37	-5	0.001

Table 4. Summary of results Levene test for equality of variances

Variables		F	Significance level
Psychological needs	Autonomy	0.16	0.68
	Competence	0.33	0.56
	Dependence	0.03	0.86
Perfectionism	Positive	0.58	0.29
	Negative	0.17	1.85
	Perception of body image	0.74	0.10

be said that there is no significance relationship between men's and women's (applicants of cosmetic surgery) psychological needs, perfectionism, and perception of body.

DISCUSSION

According to the findings of this research, the first hypothesis was confirmed, and it was concluded that there is a significant relationship between psychological needs, perfectionism, and perception of body image of cosmetic surgery applicants. In addition, from predictive variables, "negative perfectionism" factor has a more effective role in the prediction of perception of body image in cosmetic surgery applicants. This result corresponded with findings of Hass, and Von Soest (2009) (Haas, 2008; Von Soest, 2009). Results of these studies have shown that there is a relationship among cosmetic surgery applicants' psychological needs, perfectionism, and perception of body image. The same as this research that has shown that negative perfectionism feature has more share in the prediction of body image in cosmetic surgery applicants. Since perfectionism increases the anxiety level and negative perfectionism leads people to the inefficient and non-constructive behaviors, so it further strengthens the basic needs of the motivational and psychological tendency of people. In people who do not have a favorable understanding of their own appearance, choosing cosmetic surgery without a justifiable medical reason is a solution based on emotional copying style (Nerini, 2014). It seems that the main motivational factor for this action is a failure to satisfy basic psychological needs. Awareness of this discontent is possible with a focus on "self". Intellectual challenges such as how people think about us and our belief about how they will behave have important implications on how we think about ourselves. When a person expects to be rejected for the sake of some of her features, she can choose between several different possible answers that choosing cosmetic surgery is one of these responses. Also in the second hypothesis, it was concluded that there are differences between men's and women's (cosmetic surgery applicants) psychological needs, perfectionism, and perception of body.

On the basis of findings of this research, the fourth hypothesis was not confirmed and it was concluded that there is no significant difference among psychological needs, perfectionism and perception of the body of samples. This result corresponds with study results of Pasha, Naderi and Akbari (2009) and sarwer (2005) (Naderi, 2008; Sarwer, 2005). Basic psychological requirements and perfectionism characteristic of people, over time and in the process of mental transformation, leading to the formation and evolution of the cognitive, emotional and behavioral characteristics. Accordingly, these factors will create inefficient perceptions of people toward themselves and their environment and will have concerns and conflicts about their appearance in the future. Therefore, men and women who were applicants of cosmetic surgery, in most cases did it influenced by the same motivational factors (Webb, 2015).

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Effect of water stress on gland function and some qualitative traits of commercial cultivars and promising potato clones

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ABSTRACT

In order to evaluate the effect of water stress on gland function and some of qualitative traits of commercial cultivars and potato clone, the experiment was performed as split-plot design based on randomized complete blocks with 3 replications in agricultural research and education station and natural resources of Ardabil in 2015. In order to determine the tolerance of cultivars and clone against water stress, susceptibility indexes and tolerance against water stress were used. The results of analysis of variance showed that the traits of total gland function, gland protein function, and percentage of dry matter of the gland, under the effect of irrigation levels and all traits of interest, became significant under the main effect of genotype. Also, the interaction of irrigation levels and cultivar was significant for the percentage of the dry matter of the gland. In this study, substituting moderate water stress for normal irrigation lead to about 27% saving on irrigation water consumption. Agria and Marfona cultivars produced highest percentage and function of protein and gland methionine. Clone 397008-9 showed the highest function of total gland and according to GMP, MP, and SSI indexes, had higher tolerance threshold relative to water stress. Also, Agria and Spirit cultivars had highest percentage of dry matter in severe and moderate water stress, respectively. STI showed significant superiority in identifying resistant genotypes.

KEY WORDS: TOLERANCE INDEX, PROTEIN FUNCTION, DRY MATTER, METHIONINE

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is a one-year plant from Solananceae family and after corn, rice, and wheat, is in the fourth place in global scale. In this scale, Iran is in the twelfth place regarding potato production and in the Asia, is in the third place, so that the cultivation area of this product during 92-93 was 159000 hectares (FAQ, 2014). Since the provision of food necessitates maximum utilization of limited resources, therefore, awareness of factors that influence the function of each plant and its effect on increased agricultural plants function is very important. Also, its provision is an important factor in increasing the quality and quantity of crops, especially potato. Water stress is one of the most important factors that limits crops production (Passioura, 2007). Numerous studies have shown that potato has high susceptibility to water stress in all stages (Rezazadeh et al., 2015; Shock et al., 2013, Li et al., 2016).

The results of studies by Haghighi et al. (2015) showed that the effect of water stress treatments on gland function is significant and with increased irrigation water, the function increases. Water stress has a direct effect on proteinizaton process and water shortage in plant, in addition to hydrolyzing the available proteins, stops synthesis of new proteins (Kazemi, 1994). Since the function of crops under stress condition, due to genotype and environmental effects, is not considered as a suitable benchmark to select genotypes resistant against drought, various indexes are proposed to select plants based on function. In investigating the tolerance of water deficit under in vitro and in vivo conditions, Hasanpanah (2010), based on MP, GMP, STI, and MSTI, selected Kaiser as the superior cultivar under normal and water stress conditions. Since a major part of lands are under arid and semi-arid conditions and rainfall are decreased in recent years, potato production has been faced by numerous problems. Therefore, accurate analysis as well as qualitative and quantitative assessments and determining to tolerance and susceptibility against water in different growth stages constitute one of the most important methods to decrease concerning effects of water deficit. Therefore, this study aimed to use tolerance and susceptibility against water stress in farm conditions.

MATERIAL AND METHODS

This study was conducted during 2014-2015 in agricultural research and education station and natural resources of Ardabil. Ardabil province has moderate and semi-temperate climate with very cool winters and springs and moderate summers. Average maximum and minimum annual temperature and maximum regional temperature were 19.8, 15.18, and 21.58, respectively. Also, average rainfall has been reported as 310 mm (Unknown, 2016). Soil properties of the area are presented in Table (1).

The experiment was performed as split-plot design based on randomized complete blocks with 3 replications. Irrigation as the main plot was considered with three levels including provision of 100% water needs (control treatment), moderate water stress, and severe water stress. Also, cultivar was considered as the minor plot at six levels including Agria , Spirit, Marfona , Luca, Hermes, and promising clone. In the autumn of 2014, land preparation operation was performed as deep plowing, disc cutting, and land levelling the farm. At the beginning of May, stacking and plotting operation was performed and glands were cultivated. Treatments were implemented in experimental plots with the area of 22.5 square meter consisted of four 6-meter lines with the distances of 75 cm between two rows and 25 cm between two bushes. To prevent water penetration from adjacent plots, 3 m and 1.5 m were considered between the main plot and the minor plots as margin. To control Colorado beetle pest, confidorous pesticide (250 ml) was used before flowering of potato. Also, to prevent whippery, mancozeb was used (1 kg/ha) before flowering stage. According to the results of soli analysis in the area of interest, 150 kg/ha ammonium phosphate was used in two turns (50% while cultivation and 50% in gland formation), urea (350 kg/ha) in three turns (25% while cultivation, 50% while emergence, and 25% immediately after gland formation), and potassium sulfate (150 kg) in one turn (cultivation). Weed practices were performed in two turns before gland formation in all experimental plots. In Ardabil and neighborhood areas, water stress mainly occurs in July and August and for this reason, the purpose is to identify cultivars that are resistant against stress in the final tension of the season. The implementation of irrigation treatments was as follow:

Normal irrigation treatment was initiated one day after gland cultivation (Juan 6) and continued until October, 2 (harvesting) that was accompanied by two irrigations (5-7 days) with providing 100% water needs of the plant. In the plots under this treatment, irrigation times from cultivation to harvesting, included 11 times and water volume and applied water were 10950 and 11638 cubic meter, respectively.

Moderate water stress treatment was initiated one day after gland cultivation (Juan 6) and continued until July, 2 with irrigation period of 5-7 days and provision of 100% water need. Then, the irrigation operation stopped after 15 days (two times of irrigation). After this period, irrigation operation followed normal condition until October, 2 with the provision of 100% water need.

Therefore, in the plots of this treatment, the number of irrigations until cultivation was 9 and water volume and applied water were estimated as 8040 and 8728 cubic meter, respectively. Severe water stress was initiated one day after cultivation (Juan 6) and continued until July, 2 with the provision of 100% water need and irrigation period of 5-7 days in normal condition. Then, the irrigation operation continued until October, 2 win normal condition and provision of 100% water need. Therefore, in the plots of this treatment, the number of irrigations was 7 and irrigation water and applied water were 6600 and 7288 cubic meter, respectively.

The initiation of irrigation in the farm of interest in climatic condition of Ardabil was estimated based on evaporation of 28 mm water from pan evaporation surface (40% humidity discharge of available water). In other words, to estimate water in experimental plots, data related to evaporation from pan evaporation (Class A) were used. Also, Relationship (1) was used to estimate the required water for normal irrigation treatment (provision of 100% required water).

Relationship 1: $S \times EP \times 8/0 = IW$

Here, IW is required water (cubic meter), 0.8 is pan coefficient (Moradi et al., 2000), EP is evaporation from pan evaporation (mm), and S is the area of experimental plot (square meter). Then, in each irrigation turn, the required water for plots was estimated and directed to the plots of interests. Irrigation operation was performed for all plots until 75 days after cultivation and after that, moderate and severe water stresses were implemented. To measure daily precipitation, rainfall meter device was installed at the pan evaporation and each day in certain time (12 PM), measurements were performed. Comparison of the water required for each treatment and rainfall has been presented in Table (2).

Harvesting was performed by Octoer, 2. For this purpose, by the end of the season, to measure gland function, sampling was done for 2 central row in each plot (5 m) and to remove marginal effects, to marginal rows were removed. Then, the harvested glands from each surface (5 ×1.5m) were weighted and the resulted number was determined as the total gland function at the surface level (hectares). To determine the dry matter percentage of the glands, first, 10 glands were randomly selected and from each samples, 200g was sliced and after placing them within paper envelopes, they were placed inside the oven for 48h at the temperature of 72 C (Hasanpanah & Hoseinzadeh, 2007). After this period, samples were weighted by digital balance and the dry matter percentage was estimated using Relationship (2):

Relationship 2: dry matter percentage – (gland weight after drying/initial weight of glands) ×100

The multiplication of gland dry matter percentage and gland function has been considered as the gland dry matter function in hectares. To determine the weight of gland protein, Bradford method (1976) was used and to estimate protein function, relationship (3) was used:

Relationship 3: gland function×100/gland protein percentage – protein function. Also, methionine function (having data related to gland protein percentage and function) was estimated using Relationship (4):Relationship 4: methionine function - protein function×gland methionine percentage/100

In this study, after harvesting and estimating genotypes in both stress and normal conditions, five main indexes were used to determine tolerance and susceptibility of genotypes against water stress. To define different indexes, following terms have been used:

Yp: potential function of each genotype in stress-free environment
Ys: potential function of each genotype in stress environment
$\bar{Y}p$: average function of all genotypes in stress-free environment
$\bar{Y}s$: average function of all genotypes in stress environment

Stress Susceptibility Index (SSI) was proposed by Fischer and Mourer (1978) as follow:

Relationship 5: $SSI = [1- (Ys /Yp)] /SI$

In this relationship, SI is estimated as follow:

$SI = 1- (\bar{Y}s/\bar{Y}p)$

In genotype assessment using SSI, higher value of the index shows genotype susceptibility to stress. Therefore, genotype selection should be based on low SSI values.

Tolerance index (TOL) was defined Roseili and Hambline (1981) as function disorder in stress and stress-free environments:

Relationship 6: $TOL = Yp – Ys$

In genotype assessment using this index, higher index value shows susceptibility to stress; therefore, genotype selection should be based on low value of TOL.

Mean Productivity (MP) that was proposed by Rosielle and Hamblin (1981) as follow:

Relationship (7): $MP = (YS + YP)/2$

This index in the mean of genotype utilization in stress and stress-free conditions and selects genotypes that have high function in desirable conditions, but are in undesirable condition in term of function. Therefore,

selection based in MP index is efficient for genotypes with high potential.

Stress tolerance Index (STI) is estimated by Relationship (8) and Geometric Mean Productivity (GMP) is estimated by Relationship (9):

$$\text{Relationship (8): STI}=\frac{yp \times ys}{(\bar{y}p)^2}$$

$$\text{Relationship (9): } GMP=\sqrt{(Yp)(Ys)}$$

To perform statistical analyses and data estimations such as analysis of variance, correlation coefficient between assessment characteristics, and estimating tolerance indexes, SAS 9.1 was used. Also, to compare means, LSD test at the probability level of 5% was used.

Table 1. Soil test results at the depth of 0 to 30 cm

Soil texture	Saturated moisture content	Electrical conductivity	pH	Organic carbon percentage	Total percentage of neutralizing material	Total nitrogen percentage	Zinc (mg/kg)	Iron (mg/kg)	Manganese (mg/kg)	Copper (mg/kg)	Absorbable potassium (mg/kg)	Absorbable phosphorus (mg/kg)
Lumi	43	0.874	0.797	1.03	6.5	0.1	10.1	5.18	16.2	7.52	318	5.8

Table 2. Comparing irrigation water volume of experimental plots

Treatment	Irrigation rounds (day)	Number of irrigations	Irrigation volume (cubic meter/ha)	Rainfall		Effective rainfall (cubic meter/ha)	Applied water volume (cubic meter/ha)
				mm	Cubic meter/ha		
Normal irrigation	5-7	11	10950	86	860	688	11638
Moderate stress	5-7	9	8040	86	860	688	8728
Severe stress	5-7	7	6600	86	860	688	7288

Table 3. Rainfall statistics of the area in 2015

Month	Rainfall (mm)	Month	Rainfall (mm)
April	35.7	October	58.3
May	26.5	November	46.6
Juan	7	December	13.9
July	3.6	January	6.4
August	0	February	24.9
September	48.9	March	36.7

RESULTS AND DISCUSSION

TOTAL GLAND FUNCTION

The results of analysis of variance for this trait showed that there is a significant difference between irrigation levels and cultivars at the probability levels of 5% and 1% while the interaction of these two factors did not show any significant difference (Table 4). Comparison of total gland function at different irrigation levels showed that normal irrigation treatment has highest function at the surface level (31290 kg/ha). Also, moderate water stress was placed in mutual statistical group and showed a significant difference with severe water stress treatment (Table 5).

According to the economic importance of gland function as well as difference in two irrigation rounds between normal irrigation and moderate water stress, it seems that the implementation of moderate water stress in potato farms and replacing it by normal irrigation method, in addition to obtaining suitable total function, leads to relative saving on water consumption. Significance of the difference between genotypes shows the variety of genetic materials for the trait of interest, so that the promising clone 397008-9 with including Agria, Spirit, Marfona, Luca, and Hermes shows the highest gland function mean and significant different with other cultivars. Also, Spirit and Hermes cultivars produced minimum total mean of gland function (Table 6).

Correlation coefficient table showed that total gland function has a positive and significant correlation with most of traits (Table 8). In moderate and severe water stress, a significant and positive correlation was observed between total gland function and gland protein function (Tables 9 and 10). Also, a significant and positive correlation was observed between gland dry matter and total gland function under moderate water stress. Also, in moderate water stress, MP and GMP, in addition to having significant and positive correlation with each other, showed the same correlation with STI (Table 11).

Table 4. Square mean of traits in potato cultivars and irrigation levels

Change sources	Degree of freedom	Total gland function	Gland protein percentage	Gland protein function	Gland dry matter percentage	Gland dry matter function	Gland methionine value
Replication	2	206.37	0.0003	2959.44	0.69	8.72	0.002
Irrigation levels	2	118.34*	0.00794**	619.92**	22.14**	1.02ns	0.00032ns
False	4	31.94	0.00017	330.04	0.43	1.11	0.002
Genotype	5	316.87**	0.01003**	4471.16**	26.32**	18.82**	0.032**
Cultivars × irrigation levels	10	35.83ns	0.00014ns	471.88ns	3.46**	1.36ns	0.001ns
False	30	41.15	0.0002	584.72	0.53	1.44	0.003
Change percentage (%)		22.59	3.99	23.38	3.75	21.59	29.10

Table 5. Comparing the mean of irrigation levels on some of potato traits

Irrigation levels	Gland protein function (kg/ha)		Gland protein percentage (%)		Gland total function (kg/ha)	
Normal irrigation	190.01	b	3.4	b	31290	a
Moderate stress	205.71	a	3.76	a	27590	ab
Severe stress	192.14	b	3.7	a	26320	b

Table 6. Comparing the mean of cultivar on some of potato traits

Cultivars	Gland methionine (microgram/mg)		Gland dry matter function (kg/ha)		Gland protein function (kg/ha)		Gland protein percentage (%)		Gland function (kg/ha)	
Agria	0.25	a	6240	a	253.21	a	4	a	29647	a
Marfona	0.25	a	6930	a	252.46	a	4	a	32403	a
Luca	0.16	b	6370	a	230.81	a	3.6	b	33100	a
Spirit	0.12	b	3900	b	146.65	b	3.2	d	22790	b
Hermes	0.14	b	3540	b	119.95	b	3.4	c	19303	b
Clone 379008-9	0.23	a	6360	a	229.21	a	3.6	b	33110	a

In severe water stress, MP and GMP showed a significant and positive correlation at the probability level of 5% with STI and under these circumstances and probability level of 1%, a significant and positive relationship was observed between MP and GMP (Table 12). According to the table of simple coefficients between indexes, correlation between potential function and stress in moderate stress (r=0.893) and severe stress (r=0.937) was significant (Table 13 and 14). The function of genotypes in stress-free environment showed a positive correlation with STI, MP, and GMP. Also, correlation between the function of genotypes under moderate water stress and STI, MP, and GMP as well as under severe water stress with STI, MP, GMP, and TOL was positive. High correlation between STI and genotype function in stress and stress-free environments shows superiority of this index relative to the indexes of interest in the assessment of genetic variety and screening tolerant genotypes as well as estimation of function resistance. Maralian et al. (2014) investigated the effect of low irrigation of gland function of different potato genotypes and found that 60% provision of water leads to decreased gland function of potato genotypes from 754 g to 640 g (17.7%).

GLAND PROTEIN PERCENTAGE

The results of analysis of variance showed that there is a significant difference between different irrigation levels and cultivars at the probability level of 1% while no significant difference was observed in the interaction of these two factors for the traits of interest (Table 4). Comparing gland protein percentage at irrigation levels showed that moderate and severe water stress treatment have highest mean of gland protein percentage and

showed a significant difference with normal irrigation (Table 5). It seems that high values of protein in water stress treatments is the result of low gland function. Significance of the difference between genotypes showed genetic variation for traits, so that Agria and marfona cultivars showed highest percentage of gland protein (4%) and significant difference with other cultivars. Also, the lowest gland protein percentage mean belonged to Spirit (Table 6). Correlation coefficients between assessment characteristics in normal irrigation showed that protein percentage has a significant and positive correlation with most of traits (Table 8). Also, although no positive and significant correlation was observed with other traits, under severe water stress, methionine level and gland dry matter percentage showed significant and positive correlation at the probability levels of 1% and 5% with traits (Tables 9 and 10). With increased water stress severity, protein percentage increases; therefore, change in protein structure or its destruction is one of the metabolic stages that may be influenced by water stress (Ommen, 1999).

GLAND PROTEIN FUNCTION

The results of analysis of variance for this trait showed that there is a significant relationship between irrigation levels and cultivars at the probability level of 1% while no significant relationship was observed in the interaction between these factors (Table 4). According to the comparison between protein function mean at the irrigation level, moderate water stress showed the highest gland protein function (205.71 kg/ha) and showed a significant difference with normal irrigation and severe water stress. Lowest gland protein function was observed under normal irrigation condition and severe water stress (Table 5). In other words, by replacing moderate water stress with normal irrigation, about 27% of water consumption was saved. Significant difference between genotypes showed genetic variety between cultivars. Comparing the effect of genotype on gland protein function showed that the highest mean of gland protein function is related to Agria , Marfona , Luca, and promising clone 379008-9. Lowest mean of protein function at the surface level belonged to Spirit and Hermes (Table 6). Correlation coefficient table about traits in normal irrigation showed that gland protein function has a significant and positive relationship with most of traits (Table 8). Also, under moderate and severe stress conditions, significant and positive correlation was observed between this trait and gland function. Moreover, the trait and gland dry matter function in moderate and severe water stress conditions showed significant and positive correlation at the probability levels of 5% and 1% (Tables 9 and 10).

GLAND DRY MATTER PERCENTAGE

The results of analysis of variance showed that there is a significant different between different moisture contents, cultivars, and interaction between irrigation lev-

Table 7. Gland dry matter percentage of potato cultivars at different irrigation levels

Irrigation treatments	Clone 397008-9		Hermes		Luca		Spirit		Marfona		Agria	
Normal irrigation	19	bcd	16.25	bcd	16.67	bcd	16.82	bcd	18.65	bcd	19.76	abc
Moderate water stress	18.85	bcd	19.02	bcd	17.34	bcd	21.75	a	20.59	ab	21.42	ab
Severe water stress	19.86	bcd	19.57	bcd	17.32	bcd	19.54	bcd	19.19	bcd	22.88	a

Table 8. Correlation coefficient between traits in normal irrigation conditions

	Methionine level	Protein function	Protein percentage	Gland dry matter function	Gland dry matter percentage	Gland total function
Gland function						-
Gland dry matter percentage					-	0.79*
Gland dry matter function				-	0.90**	0.97**
Protein percentage			-	0.96**	0.91**	0.96**
Protein function		-	0.96**	0.94**	0.80*	0.94**
Methionine	-	0.81*	0.92	0.90**	0.99**	0.78*

Table 9. Correlation coefficients between traits under moderate water stress

	Methionine level	Protein function	Protein percentage	Gland dry matter function	Gland dry matter percentage	Gland function
Gland function						-
Gland dry matter percentage					-	0.34
Gland dry matter function				-	0.76	0.87*
Protein percentage			-	0.38	0.51	0.19
Protein function		-	0.34	0.84*	0.37	0.95**
Methionine level	-	0.41	0.49	0.78	0.99**	0.37

Table 10. Correlation coefficients between traits under severe water stress

	Methionine level	Protein function	Protein percentage	Gland dry matter function	Gland dry matter percentage	Gland function
Gland function						-
Gland dry matter percentage					-	0.26
Gland dry matter function				-		0.80
Protein percentage			-	0.75	0.99**	0.26
Protein function		-	0.61	0.95**	0.32	0.98**
Methionine level	-	0.31	0.80*	0.55	0.83*	0.55

Table 11. Estimating tolerance and susceptibility of cultivars and clone to water stress by indexes in moderate water stress

Cultivars and clone	MP	SSI	STI	GMP	TOL	YS	YP
Agria	31070	2.16	0.684	30520	11650	26810	36890
Marfona	34530	2.15	0.807	34330	7380	28150	38220
Spirit	34330	0.51	0.954	34320	1610	30630	35140
Luca	22220	0.62	0.944	22210	1280	23930	22860
Hermes	34620	0.09	-17.28	17340	50	23080	17440
397008-9	35670	0.09	-14.00	21670	7670	33000	37000

Table 12. Estimating tolerance and susceptibility of cultivars and clone to water stress by indexes in severe water stress

Cultivars and clone	MP	SSI	STI	GMP	TOL	YS	YP
Agria	31850	2.29	0.727	31450	10090	25240	36890
Marfona	33800	6.72	0.736	32800	10070	30840	38220
Spirit	32890	3.27	0.872	32810	4500	33530	35140
Luca	23400	-1.19	1.047	23390	1070-	21580	22860
Hermes	20260	-8.23	1.323	20060	5630-	17390	17440
397008-9	35000	2.76	0.892	34940	4000	29330	37000

Table 13. Correlation coefficients between susceptibility indexes and tolerance to drought and function in moderate water stress

	YP	YS	TOL	GMP	STI	SSI	MP
YP	1						
YS	0.893	1					
TOL	-0.051	-0.496	1				
GMP	0.964	0.980	-0.315	1			
STI	0.952	0.988	-0.353	0.999*	1		
SSI	0.319	-0.712	0.963	-0.559*	-0.593	1	
MP	0.969	0.977	-0.297	1*	0.998*	-0.544	1

els and cultivars at the probability level of 1% (Table 4). Comparing the effect of irrigation levels on gland dry matter percentage showed that the highest mean of gland dry matter is related to moderate and severe water stress conditions and these treatments were at the highest level of statistical group and showed a significant difference with normal irrigation treatment (Table 5). It seems that increased dry matter percentage of potato under water stress is resulted from low level of gland function. According to considering water stress treatments in the same group and necessity for savings on water consumption, moderate water stress is recommended for nutritional goals and potato processing (chips, franchise, etc.). Significant difference between genotypes showed the existence of genetic variation between cultivars. Comparing the effect of this cultivar on this trait showed that Agria and Spirit have the highest mean of gland dry matter and showed a significant difference compared with other cultivars. Hermes with the lowest mean of gland dry matter was at the lowest position of the statistical group (Table 6).

Comparing the mean of interaction between irrigation levels and cultivar for this trait showed that Agria and Spirit have the highest percentage of gland dry matter under severe (22.8%) and moderate (21.75%) water stress conditions and it seems that it can be used as a suitable mixture to produce fried products and use in processing industry to save water consumption in agriculture. Correlation coefficient table showed that gland dry matter percentage has a significant and positive correlation with most of traits (Table 8). Also, this trait under moderate water stress showed a significant and positive correlation with methionine level and under severe stress condition, showed a significant and positive correlation with methionine and gland protein percentage (Tables 9 and 10).

GLAND DRY MATTER FUNCTION

The results of analysis of variance for this trait showed that there is a significant difference between cultivars at the probability level of 1% while irrigation levels and interaction between these factors did not show any significant difference (Table 4). Significant difference between genotypes shows the variety of genetic substance of cultivars for the trait. Comparing the effect of cultivar on this trait showed that Marfona wit Luca, clone 397008-9, and Agria have the highest gland dry function mean and showed a significant difference with other cultivars. In this study, Spirit and Hermes showed the lowest mean of gland dry matter function at the surface level (Table 6). Correlation coefficient table between traits in normal irrigation condition showed that gland dry matter percentage has a significant and positive correlation with most of traits (Table 8). Also, this trait under moderate water stress showed a significant and

Table 14. Correlation coefficient between susceptibility indexes and tolerance to drought and function in severe water stress

	YP	YS	TOL	GMP	STI	SSI	MP
YP	1						
YS	0.937	1					
TOL	-0.528	0.792	1				
GMP	0.973	0.992	-0.710	1			
STI	0.960	0.997*	-0.745	0.999*	1		
SSI	0.289	-0.972	0.912	-0.936**	-0.953*	1	
MP	0.978	0.989	0.695	1**	0.997*	-0.928	1

positive correlation with gland and protein function (Tables 9 and 10). The results of studies by Kumar et al. (2007) with different irrigation treatments showed that increased water stress decreases gland dry matter function.

GLAND METHIONINE LEVEL

Analysis of variance for the trait of interest showed that there is not any significant difference between different irrigation levels and interaction between irrigation and cultivar levels while cultivars for this trait showed a significant difference at the probability level of 1% (Table 4). Significant difference between genotypes showed the variety of genetic substances for the trait of interest. Comparing the mean of the effect of cultivar on this trait showed that the highest methionine level belongs to Agria , Marfona , and clone 3977008-9 and showed a significant difference with other cultivars. Luca, Hermes, and Spirit produced lowest level of gland methionine (Table 6). Correlation coefficient table between traits in normal irrigation condition showed that gland methionine has a significant and positive correlation with most of traits (Table 8). Also, this trait, under moderate water stress, showed a significant and positive correlation with gland dry matter percentage and under severe water stress, showed a significant and positive correlation with gland dry matter percentage and gland protein percentage (Tables 9 and 10). Muttucumaru et al. (2015) by investigating 5 cultivars of potato resistant and susceptible to drought found out that cultivars were significantly different in terms of methionine concentration. Also, methionine concentration of cultivars resistant to drought is higher than cases sensitive to drought.

CONCLUSION

Moderate water stress lead to the production of suitable gland function. Also, the highest percentage of gland protein (3.76%), gland protein function, and highest dry matter percentage (19.83) belonged to this treatment. Therefore, due to the economic importance of gland function and decreased levels of water consumption (2910 cubic meter) compared with normal treatment, the implementation of moderate stress and replacing it with normal irrigation seems more economic. Agria and Marfona produced the highest protein percentage (4%) and gland methionine. Also, Agria and Spirit in severe (22.88%) and moderate (21.75%) water stress conditions showed highest gland dry matter percentage and it seems that it can be introduced as a suitable treatment. The highest values of gland protein function belonged to Agria and Marfona . Clone 397008-9 produced the highest gland function mean ((33100 kg/ha) and according to MP indexes (severe and moderate water stress), SSI (moderate water stress), and GMP (severe water stress) showed higher tolerance compared with water stress and it can be used for commercial purposes. High correlation between STI and genotype functions in stress and stress-free environments showed the superiority of this index in screening tolerant genotypes and function tolerance estimation.

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Interleukin-17 concentration as a biomarker in diagnosis of exudative pleural effusion compared with benign pleural effusion

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ABSTRACT

Pleural effusion is one of signs and complications resulting from malignant disease such as lung and breast cancer, and also tuberculosis and infective lung disease by cytological analysis of pleural fluid we can use of tumor marker and other biomarkers to better diagnose malignant pleural effusion.in this study we examined the concentration of interleukin-17 in pleural fluid with causes of exudative pleural effusion in the patients referred to hospital of 2015-2016.This is a descriptive-analytical and case-control study and 130 patients with exudative pleural effusion were enrolled in the study after an informed consent samples collected from the patients divide into two main group including 88 patients with malignant pleural effusion and 42 patient with benign effusion. in the next step by using of the same previous pleural fluid samples, the concentration of interleukin-17 was measured with ELISA by specific kit after entering to computer through SPSS-18 statistical software, description of data was done into frequency and percentage.Interleukin-17 concentration was (69.73±64.58) in patients with malignant causes and (55.32±43.60) in benign causes.The results showed that this different was statistically significant (P=0.02) and interleukin -17 rate, is higher in the malignant pleural effusion.According to higher levels of interleukin-17 in malignant pleural effusion maybe we can achieve important result in differentiating between malignant and non-malignant pleural exudate, without the need for invasive procedures, by putting together the clinical symptoms, the interleukin-17 concentration in pleural fluid and pleural fluid cytology results.

KEY WORDS: INTERLEUKIN-17, EXUDATIVE PLEURAL EFFUSION, MALIGNANT PLEURAL EFFUSION, BENIGN PLEURAL EFFUSION, BIOMARKER

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INTRODUCTION

Pleural effusion is an excessive accumulation of fluid in the pleural space, which affects annually about one million people around the world. There are a range of causes for the disease; benign diseases such as infections, heart and liver failures, rheumatic diseases and drugs on the one hand and fatal cancers of lung and other visceral organs on the other hand are placed in this spectrum (Esther et al 1997).

Plain chest pleurography is a simple diagnostic measure to detect pleural effusion, which appears as flattening and displacement between costophrenic angles. With the presence of less fluid or abnormal localization, ultrasound or CT scan can be used to conduct pleural tap (Heffner et al 1997, Light et al 1972, Ryland et al1998). Cytologic analysis of pleural fluid is the most common method for diagnosis of malignancy; while the specificity of cytologic findings is 100%, unfortunately only about 60% of malignant effusions can be detected through this technique (Alema'n et al 2010). For undiagnosed exudative effusions with suspicion of malignancy but negative cytology, more invasive approaches are necessary (Hooper et al 2010 Chun Hua et al., 2017).

Closed pleural biopsy has less additional diagnostic value and thoracoscopy is the preferred method, since it is diagnostic in 90% of patients (Light 2006, Roberts 2010 and Neragi-Miandoab 2006). However, there is no possibility of access to this invasive procedure in all centers. Many investigations have assessed the ability of tumor markers and other biomarkers to improve the diagnosis of MPE (Botana-Rial et al 2011, Kremer et al 2010).The combined use of different markers has also been proposed in some studies (Kremer et al 2010, 2013).

Unfortunately, none of these markers has shown the sensitivity and specificity enough to select as a diagnostic marker of MPE (Hooper et al 2010). In a study, a type of chemical safety approach has been used to search for non-invasive markers of lung cancer in PE (Porcel et al 2004). In this regard, several chemical biomarkers of inflammation were found that were differentially expressed in MPE vs. BPE. Among these biomarkers, IL-17 and CEA were expressed at higher quantities in MPE than BPE. Interleukin 17 is a potential inflammatory cytokine produced by Th17 cells; it is expected to be used as a marker for the diagnosis of pleural effusion. The aim of this study is to determine the concentration of IL-17 in pleural fluid to facilitate differentiation between MPE and BPE.

MATERIALS AND METHODS

The Deputy of Science and Technology of Golestan University of Medical Sciences and Research Ethics Committee approved study design and implementation protocol. All actions taken, had no physical, psychological or financial losses for the participants in the research. This descriptive and analytical study was performed on 130 patients with exudative pleural effusion who were admitted to Shahid Sayyad Shirazi Teaching Hospital in Gorgan during 2015-2016, and enrolled in the study after obtaining informed consent.Routine diagnostic procedures such as X-ray, CT scan and ultrasound, if necessary, were performed for all patients before starting the study. After history taking and exact physical examination, thoracentesis was carried out to prepare pleural fluid samples that were frozen at -20°C after centrifugation for 15 min.

The samples were analyzed for biochemical properties, pH, glucose and proteins; accordingly, exudative pleural effusion was differentiated from transudative type and patients with transudative pleural effusion were excluded from the study. Malignancy of pleural effusion was diagnosed according to our and previous studies using VATS technique; such that, after inserting VATS instruments through 1 to 2 incisions and after viewing the parietal and visceral pleura as well as evidence of metastases, biopsy was performed from the parietal pleura and pleural fluid for cytology testing. The samples were sent to the pathology laboratory for detecting malignancy and its type.

In terms of etiology and based on the findings of the gold standard of pleural fluid analysis (microbiology and cytology testing, including cell differential count), exudative pleural effusion were classified into two main MPE and BPE groups. According to previous studies, since between 40 and 50 percent of the cases showed malignant exudative pleural effusion, so the samples were divided almost with the same proportion. At a later stage, by using the same previous pleural fluid samples, the IL-17 levels were measured via ELISA kit according to the manufacturer's protocol and the samples were stored at -80°C until testing.Having pleural effusion disease was inclusion criterion. Exclusion criteria included transudative pleural effusion, diabetes, autoimmune diseases and rheumatic diseases.

Data were analyzed using statistical SPSS V.18 software. Normal distribution and data homogeneity were assessed using the Kolmogorov-Smirnov test.

In addition, the mean IL-17 level was compared between the two groups using independent t-test. Mann-Whitney test was applied for normal distribution of IL-17 concentration. Significance level of the tests was 0.05.

RESULTS AND DISCUSSION

After examining, 130 patients were divided into two groups of malignant pleural effusion (n=88, 67.7%) and

benign pleural effusion (n=42, 32.3%) with a mean age of 57 and 59 years respectively. There was no statistically significant difference between IL-17 level and age of the patients. The patients in MPE group included 44 males (50%) and 44 females (50%), but the patients in BPE group consisted of 26 males (61.9%) and 16 females (38.1%).

In the MPE and BPE groups, 41 and 21 people were smokers respectively. Among the patients in MPE group, 22 (25%) had primary lung cancer, 31 (35.2%) secondary breast cancer, 11 (12.5%) secondary esophageal cancer, 11 (12.5%) metastatic cancer and 13 (14.77 %) secondary stomach cancer.

The IL-17 level was 36.43±56.719 in males and 74.838±76.42 in females; and as a result of statistical analysis, no significant difference was observed for IL-17 levels in pleural fluid between males and female. Analysis of the mean pleural fluid protein levels was respectively 4373.8± 419.3 mg and 4411.7 ± 493 mg in the patients of MPE and BPE groups. There was a correlation between IL-17 and pleural fluid protein levels, but it was not statistically significant (r = 0.15). The mean IL-17 level was compared in smokers and nonsmokers, and statistical analysis showed no significant difference (P = 0.35). The results showed that 66 patients (50.8%) had a history of previous malignancy; 31 (46.97%) secondary to breast cancer, 11 (16.67%) secondary to esophageal cancer, 11 (16.67%) secondary to metastasis due to other causes and 13 (19.7%) secondary to stomach cancer had experienced MPE, and the mean IL-17 level was 63.9±36.6 in these subjects. In people who had no history of previous malignancy, including 22 (34.38%) with primary lung cancer and 42 (65.63%) with TB (totally 64 patients, 49.2%), the mean IL-17 level was 66.2 ± 75.5 in these people. Considering the higher mean IL-17 level in the group with no history of previous malignancy compared with the first group as well as P= 0.001, no statistically significant difference was observed for IL-17 levels between the two groups. The IL-17 level in pleural fluid of patients with MPE and BPE was respectively 69.73± 64.58 and 55.32±43.60; there was a statistically significant difference between the two groups (P = 0.02).

Differentiating between benign pleural effusion (BPE) and malignant pleural effusion (MPE) has remained controversial as a diagnostic issue. The majority of malignant pleural effusion (90% to 97%) is exudative type that occurs because of pleural membrane damage (Esther et al 1997). MPE can be seen as a complication in most malignancies, particularly in breast and lung cancer, while lung infections and tuberculosis cause the development of BPE.The gold standard for diagnosis of malignant pleural effusion is clinically the presence of malignant cells in pleural cells. Difficulty in differentiating between malignant and benign is the negative result of malignant cells in pleural fluid cytology. In these circumstances, differentiation from benign is problematic and the need for invasive measures such as thoracentesis is essential for the patient.

Closed pleural biopsy has less additional diagnostic value and thoracoscopy is the preferred method, since it is diagnostic in 90% of patients. However, there is no possibility of access to this invasive procedure in all centers. According to this issue, 130 patients with exudative pleural effusions were examined in this study. In the present study, 88 patients with malignant pleural effusion were compared with 42 patients with benign pleural effusion in terms of pleural fluid levels of IL-17. The patients were also evaluated for age, gender, history of previous cancer, smoking and pleural fluid protein levels. The mean age of subjects was close to each other in two age groups and no significant difference was found between the two groups. In addition, there was no statistically significant difference between IL-17 level and age of the patients.

In 2014, Chun Hua et al. examined a new biomarkers of interleukin 17 among 123 patients with exudative pleural effusion to determine the causes of pleural effusion. They showed that IL-17 level was significantly higher in MPE group compared with BPE group, similar to our results, and also stated that IL-17 can be used as a biomarker to differentiate MPE from BPE.

Since only one study has so far examined the level of IL-17 in pleural fluid, in addition to our study; so in the following discussion, we will consider similar studies closer to the present study, (Chun Hua et al., 2017).

Wang et al. in 2013 examined the levels of superoxide dismutase (SOD) in TPE and MPE, which was markedly higher in the TPE than the MPE. The results showed that SOD is not a suitable biomarker for these two types of pleural effusion (Xin-Feng Wang et al, 2013) In 2014, Chun Hua et al. examined serum levels of IL-17 in 128 patients with Non-small cell lung cancer (NSCLC). The results showed that higher levels of IL-17 in NSCLC group compared with the control group, which can be applied as diagnostic and prognostic value in patients with NSCLC.

Many studies have been conducted in the field of diagnostic value of cytology and the diagnostic value for diagnosing malignancies have been reported up to 70% in Iran. More invasive procedures such as biopsy, thoracoscopy or thoracotomy despite high sensitivity are not accepted through patients and physicians for diagnosis of tuberculosis and malignancies.

Therefore, researches in recent decades have led to find markers in pleural effusion and blood plasma to differentiate between tuberculosis and malignancies without invasion and with high-value and effectiveness of diagnosis and differentiation.

So far, only one study has examined the level of IL-17 in pleural effusion and high sensitivity has been

able to differentiate MPE than BPE. Like what mentioned above, other studies have been done on other biomarkers that some of them have been useful for the study. In our study, according to the significant differences for IL-17 levels in the two groups, its usability can be seen to differentiate between MPE and BPE. In addition, since the patients with primary lung cancer were in the group with no history of previous malignancy and the mean IL-17 was higher in these patients than in patients with secondary cancer as well as than TB, so significant difference was found between the two groups regarding history of previous cancer, though given limited studies done in this area and the lack of defined cut-off points, it definitely cannot be used as the only reference and diagnostic methods. Nevertheless, due to the apparent significant difference between the two groups, there is the possibility of using IL-17 beside other methods for differentiating malignant from benign diseases.

CONCLUSION

The results obtained from the present study demonstrated that age, gender, smoking, history of previous cancer and pleural effusion protein levels have no significant effect on IL-17 level. The IL-17 level was significantly higher in pleural effusion caused by malignant diseases than in exudative pleural effusion caused by benign diseases, especially tuberculosis.

Since the differentiation between malignant pleural effusion and benign pleural effusion, especially tuberculosis, sometimes needs to invasive procedures such as pleural biopsy or thoracoscopy, so it can be concluded that the IL-17 level regarding the significant difference can be used as a diagnostic approach in differentiating between the two types of pleural effusion.

It is recommended that the similar study should be conducted in different centers with larger sample size, as well as a variety of malignant and benign diseases can be studied separately.

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Prevalence of osseous changes of the temporomandibular joint in CBCT images of patients with and without temporomandibular disorders

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ABSTRACT

Radiographic examinations are a part of routine clinical examination of temporomandibular disorders (TMD) to verify degenerative bone changes in the joint structures. Assessment of the prevalence of osseous changes of the temporomandibular joint (TMJ) in CBCT images of the patients with and without temporomandibular disorders.CBCT images of temporomandibular joint were taken in 62 patients with temporomandibular disorders and 62 patients without TMD. The presence of bone changes including flattening, erosion, subcortical sclerosis, osteophyte, subcortical cyst, condylar hyperplasia and condylar hypoplasia of temporomandibular joint were studied at left and right sides on CBCT images. Furthermore, clinical findings in relation to temporomandibular disorders in patients were obtained from their records. The prevalence of bone changes and clinical findings in the 2 group of patients were analyzed. Radiographic findings in the right TMJ of TMD patients, included erosion (27.4%), osteophyte (17.7%), subcortical sclerosis (16.1%), condyle hyperplasia (6.5%) and flattening (40.3%). The prevalence of these bone changes in the right TMJ of non-TMD patients were 35.5, 6.5, 3.2, 0 and 37.1%, respectively. In the left side of TMD group; erosion was found in 29.0%, osteophyte 12.9%, subcortical sclerosis 12.9%, condyle hyperplasia 6.5% and flattening in 37.1% of the patients. The incidence of these changes in the same side of non- TMD group was 22.6, 3.2, 1.6, 0 and 32.3%, respectively. Significant differences were found for osteophyte incidence in the left TMJ(P=0.04), subcortical sclerosis in the right TMJ(P=0.02), subcortical sclerosis in the left TMJ(P=0.02) and condylar hyperplasia in both joints (P=0.04) between TMD and non-TMD patients.The most prevalent bone changes related to temporomandibular disorders included flattening, erosion and osteophyte. The changes were highly reported for temporomandibular disorders than healthy individuals and no significant correlation was found between TMJ bone changes and the patients' age and gender.

KEY WORDS: BONY CHANGES, CONDYLE, CONE BEAM COMPUTED TOMOGRAPHY, TEMPOROMANDIBULAR JOINT, TEMPOROMANDIBULAR JOINT DISORDERS

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INTRODUCTION

Temporomandibular disorders (TMD) are well defined as classification of abnormality including masticatory muscles, osseous and ligamentous components of the temporomandibular joints (TMJ) and neurological disease of this complex. The most common clinical signs of the TMDs are joint and muscle pain, mouth-opening limitation, crepitation and clicking. TMDs are frequently associated with degenerative bone changes involving the bone structures of the temporomandibular joint (TMJ) including flattening, osteophyte, erosion, subcortical sclerosis and pseudocysts, (Dos Anjos Pontual et al. 2012 De Melo et al. 2014, Ladeira et al. 2015 and Khojastepour et al. 2017).

Knowledge about these bone changes is fundamental for correctly diagnosing the dysfunctions associated with the disease and for appropriate treatment planning (Alexiou et al. 2009). Diagnostic procedure in patients with TMJ disorders includes the background, physical examination and other diagnostic processes such as imaging of the TMJ (Ludlow et al. 2008). A panoramic radiograph is often taken to assess the general condition of the teeth and structures of the maxilla and the mandible. To obtain more detailed information about the bony structures of the TMJ, other radiographs including computed tomography (CT) scans, and magnetic resonance images (MRI) can be taken which the latter gives information about the articular disc and the surrounding soft tissues (Honey et al. 2007). A relatively new imaging technique is cone-beam computed tomography (CBCT) and enables 3D imaging of bony structures of the skull, including the mandible and the TMJ (De Boer et al. 2014 Khojastepour et al. 2017).

The diagnostic accuracy of CBCT in detecting condylar osseous abnormalities was similar to CT images, while the sensitivity of CBCT in the detection of these abnormalities was higher (Wiese et al. 2011). The CBCT is more accurate than panoramic radiographs for the assessment of bone components of the TMJ (Ladeira et al. 2015). It is reported that the diagnostic properties of the CBCT in assessing bony conditions, is better than other existing imaging techniques. However, to date limited information exists on the role of CBCT in clinical decision-making in diagnosis or management of disorders of the TMJ (Krishnamoorthy et al. 2013). So, the aim of the current study was to determine the prevalence of osseous changes of the TMJ in CBCT images of the patients with and without TMD.

MATERIALS AND METHODS

In a descriptive cross-sectional trial, CBCT images of TMJ in 62 TMD-patients and 62 non-TMD patients (older than 17 years old) were obtained from 2 radiology centers and 3 dental clinics in Tehran, Iran during 2016-2017. The presence of bone changes of flattening, erosion, subcortical sclerosis, osteophyte, subcortical cyst, condylar hyperplasia and hypoplasia of TMJ were studied at left and right sides on CBCT images. In the TMD group the clinical evidences, and their correlation with sex and age were determined. The CBCT images of changes in flattening, osteophyte, erosion and subcortical cyst is presented in figures 1-4. Furthermore,

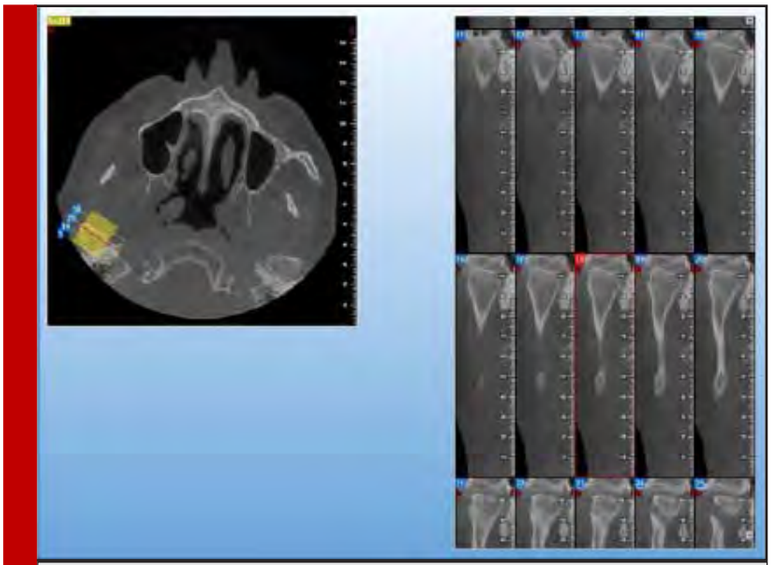

FIGURE 1. CBCT images of the changes in flattening

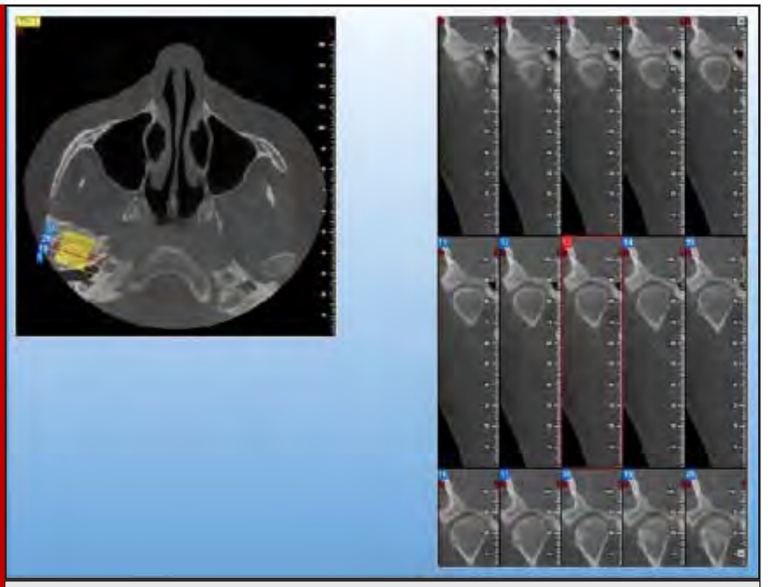

FIGURE 2. CBCT images of the changes in osteophyte

clinical findings in relation to TMD in TMD-patients were obtained from their records. The prevalence of bone changes and clinical findings in 2 TMD and non-TMD patients were analyzed using SPSS statistical software ver. 22 by Mann-Whitney U test. The correlation between bone changes and factors of age and gender were also determined by Spearman correlation ratio. $P<0.05$ was considered as significant difference.

The CBCT images of changes in flattening, osteophyte, erosion and subcortical cyst is presented in figures 1-4.

RESULTS AND DISCUSSION

In this study in the right TMJ of TMD-patients; erosion has been found in 27.4% (17 patients), osteophyte in 17.7% (11 patients), subcortical sclerosis in 16.1% (10

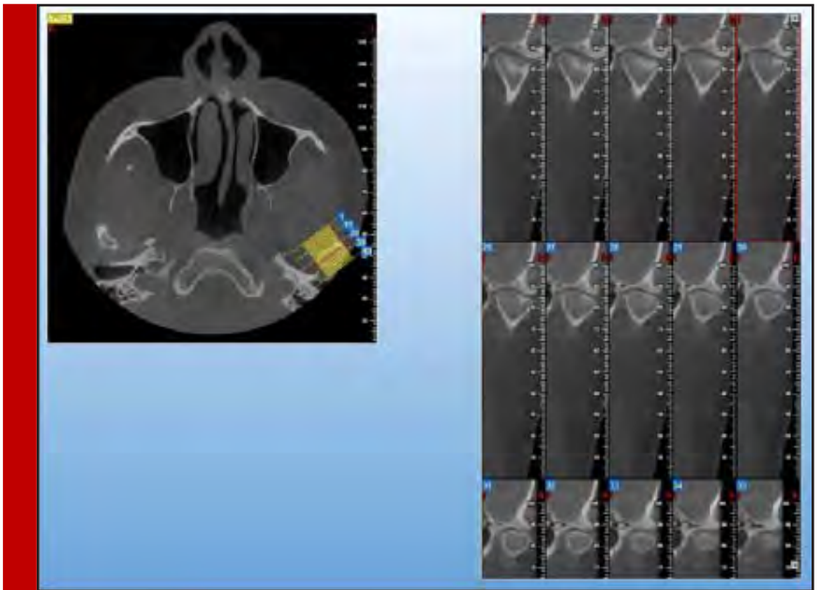

FIGURE 3. CBCT images of the changes in erosion

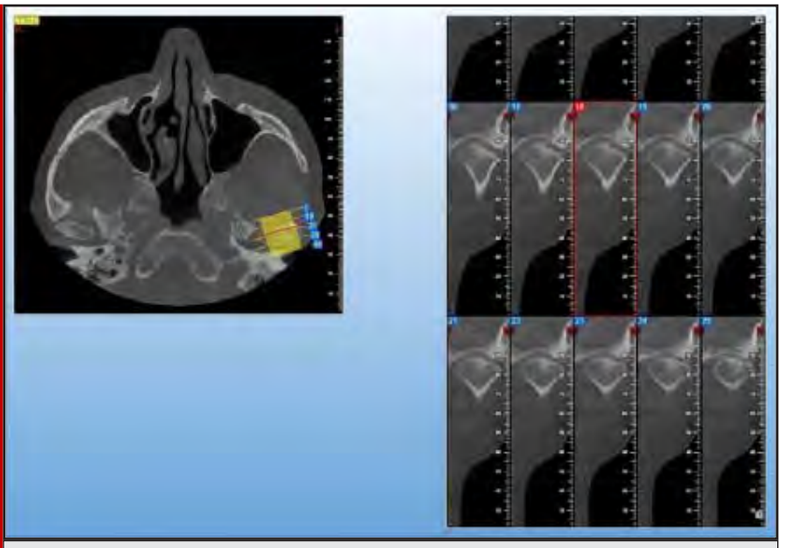

FIGURE 4. CBCT images of the changes in subcortical cyst

patients), condylar hyperplasia in 6.5% (4 patients) and flattening in 40.3% (25 patients). The prevalence of these bone changes in the right TMJ of non-TMD patients was 35.5% (22 patients), 6.5% (4 patients), 3.2% (2 patients), 0 and 37.1% (23 patients) respectively.

In the left TMJ of TMD-patients; erosion was reported in 29.0% (18 patients), osteophyte in 12.9% (8 patients), subcortical sclerosis in 12.9% (8 patients), condyle hyperplasia in 6.5% (4 patients) and flattening in 37.1% (23 patients). The incidence of these changes in the left TMJ of non-TMD patients were also 22.6% (14 patients), 3.2% (2 patients), 1.6% (1 patients), 0 and 32.3% (20 patients), respectively.

Significant differences were found regarding osteophyte incidence in the left TMJ (P=0.04), subcortical sclerosis in the right TMJ (P=0.02), subcortical sclerosis in the left TMJ (P=0.02) and condyle hyperplasia in both left and right TMJs (both: p=0.04) in TMD and non-TMD patients.

As seen in tables 1and 2, in left TMJ pain was reported in 30 (48.4%) patient and in the right TMJ in 27 (43.5%), joint tenderness was also observed in 2 (3.2%) patients.

Table 1. the frequency and percentage of pain in TMD cases and control patients

Group	Sex	right	left
	Male	7 (50%)	5 (35.7%)
Case	Female	23 (47.9%)	22 (45.8%)
	Total	30 (48.4%)	27 (43.5%)
	Male	0 (0%)	0 (0%)
Control	Female	0 (0%)	0 (0%)
	Total	0 (0%)	0 (0%)

Table 2. the frequency and percentage of joint tenderness in TMD cases and control patients

Group	Sex	right
	Male	1 (7.1%)
Case	Female	1 (2.1%)
	Total	2 (3.2%)
	Male	0 (0%)
Control	Female	0 (0%)
	Total	0 (0%)

According to table 3, muscle tenderness was observed in 4 (6.5%) patients at the right side and in 2cases (3.2%) at left.

Table 3. the frequency and percentage of muscle tenderness in TMD and control patients

Group	Sex	right	left
	Male	1 (7.1%)	1 (7.1%)
Case	Female	3 (6.3%)	1 (2.1%)
	Total	4 (6.5%)	2 (3.2%)
	Male	0 (0%)	0 (0%)
Control	Female	0 (0%)	0 (0%)
	Total	0 (0%)	0 (0%)

The deviation in opening in right and left sides were detected in 1 (1.6%) and 1 (1.6%) patients, respectively.

Table 4. the frequency and percentage of deviation in opening in case and control patients

Group	Sex	right	left
	Male	0 (0%)	1 (7.1%)
Case	Female	1 (2.1%)	0 (0%)
	Total	1 (1.6%)	1 (1.6%)
	Male	0 (0%)	0 (0%)
Control	Female	0 (0%)	0 (0%)
	Total	0 (0%)	0 (0%)

Limitation in right and left sides was observed in 13 (21%) patients (table 5).

Table 5. the frequency and percentage of limitation in case and control patients

Group	Sex	right	left
	Male	4 (28.6%)	4 (28.6%)
Case	Female	9 (18.8%)	9 (18.8%)
	Total	13 (21%)	13 (21%)
	Male	0 (0%)	0 (0%)
Control	Female	0 (0%)	0 (0%)
	Total	0 (0%)	0 (0%)

Clicking in right and left sides was detected in 26 (41.9%) and 30 (48.4%) patients, respectively (table 6).

Table 6. the frequency and percentage of clicking in case and control patients

Group	Sex	right	left
	Male	5 (35.7%)	5 (35.7%)
Case	Female	21 (43.8%)	25 (52.1%)
	Total	26 (41.9%)	30 (48.4%)
	Male	0 (0%)	0 (0%)
Control	Female	0 (0%)	0 (0%)
	Total	0 (0%)	0 (0%)

The crepitus occurrence in left and right sides in patients were 3 (4.8%) and 1 (1.6%), respectively.

Table 7. the frequency and percentage of crepitus in case and control patients

Group	Sex	right	left
	Male	0 (0%)	0 (0%)
Case	Female	1 (2.1%)	3 (6.3%)
	Total	1 (1.6%)	3 (4.8%)
	Male	0 (0%)	0 (0%)
Control	Female	0 (0%)	0 (0%)
	Total	0 (0%)	0 (0%)

The frequency and percentage of locking in both sides and subluxation in the right side were 3 (4.8%) and 14 (22.6%), respectively. The frequency of the subluxation in the left side was 15 (24.2%) patients.

Table 8. the frequency and percentage of locking in case and control patients

Group	Sex	right	left
	Male	1 (7.1%)	1 (7.1%)
Case	Female	2 (4.2%)	2 (4.2%)
	Total	3 (4.8%)	3 (4.8%)
	Male	0 (0%)	0 (0%)
Control	Female	0 (0%)	0 (0%)
	Total	0 (0%)	0 (0%)

Table 9. the frequency and percentage of the subluxation in case and control patients

Group	Sex	right	left
	Male	1 (7.1%)	1 (7.1%)
Case	Female	13 (27.1%)	14 (29.2%)
	Total	14 (22.6%)	15 (24.2%)
	Male	0 (0%)	0 (0%)
Control	Female	0 (0%)	0 (0%)
	Total	0 (0%)	0 (0%)

The TMJ, which comprises the mandibular condyle, the inferior component, and the temporal bone forming the superior component, is one of the most complex joints in the body (Wu et al. 2012). TMDs are clinically manifested by craniofacial pain in masticatory muscles and other relevant structures, limited mouth opening, and click occurring in the TMJ. (Su et al. 2014). Approximately in 60% of the population some type of TMD including myofascial dysfunction, internal derangement or degenerative joint disease has been observed. TMD is epidemic in women between 20 and 40 years old (He et al., 2010). In the current study, in the right TMJ of TMD patients, erosion was (27.4%), osteophyte (17.7%), subcortical sclerosis (16.1%), condylar hyperplasia (6.5%) and flattening (40.3%). The prevalence of these bone changes in the right TMJ of non-TMD patients was 35.5, 6.5, 3.2, 0 and 37.1%, respectively. In the left TMJ of the TMD group; erosion was 29.0%, osteophyte 12.9%, subcortical sclerosis 12.9%, condylar hyperplasia 6.5% and flattening was 37.1%. The incidence of these bone changes in the left TMJ of non-TMD patients was 22.6, 3.2, 1.6, 0 and 32.3%, respectively. Significant differences were found for osteophyte incidence in left TMJ, subcortical sclerosis in right TMJ, subcortical sclerosis in left TMJ and condylar hyperplasia in both joints between TMD and non-TMD patients. CBCT provides

accurate and reliable linear measurements of the dimensions of the mandible and the TMJ because of its nearly 1:1 reconstruction (Honda et al. 2006).

The diagnostic accuracy of CBCT in detecting cortical erosion of the mandibular condyle is greater than that of either linear tomography or panoramic radiography. Its diagnostic properties in assessing bony conditions, therefore, seem to be similar to, or better than other imaging techniques (Hilgers et al. 2005). Until now limited information exists on the role of the CBCT in clinical decision-making in diagnosis or management of disorders of the TMJ (Krishnamoorthy et al. 2013). CBCT has several advantages over CT, such as lower cost, better access to equipment, lower radiation, and diagnostic efficacy as high as CT(but superior than panoramic radiography and linear tomography) (Barghan et al. 2012). CBCT better visualizes bony changes than CT in TMD patients, analyzing lateral slices in isolation and combining coronal and lateral slices (Honey et al. 2007). So, using CBCT imaging technique to assess condylar bone changes was considered for this study.

In a research on association between condylar bone changes revealed in CBCT and clinical dysfunction index in patients with or without TMD, Khojastepour et al. (2017) significant difference reported for the prevalence of all types of bone changes between TMD and non-TMD groups. As well as their report, evaluation of CBCT images in the present study revealed significant differences between TMD and non-TMD condyles (Khojastepour et al. 2017). In a study by Moshfeghi et al. (2012) it was reported that Flattening was the most observed abnormal finding with 16.3% prevalence. Erosion, condylar hyperplasia, concavity, bifid condyle, condylar hypoplasia and sclerosis were respectively the most common abnormal findings in this study. There were no significant differences in the prevalence of abnormal radiographic findings regarding to the patient's gender, dental status and occlusion, which was in agreement with our report.

Li et al. (2015) studied the characteristics of TMJ in patients with TMD complaints and reported that in the patients with unilateral TMJ pain or joint sounds, the vertical 60° joint space of the symptomatic side was significantly increased comparing with the asymptomatic side. It is important to note symptoms of TMJ disorders may be observed with normal TMJ and vice versa, which is possibly due to measuring method, age, gender, chewing habits and etc. The condyle and mandibular fossa might differ in shape in subjects with numerous TMD complaints, since shape and function are closely related (Li et al. 2015).

In a similar study, Honey et al. (2007) reported intra observer reliability was moderate (0.57±0.22; range, 0.34-0.78). Pan-N (0.72±0.15), CBCT (0.65±0.21) and CBCT reliability was greater than corrected angle linear tomography. The diagnostic accuracy of CBCT interactively (0.95±0.05) and CBCT statically (0.77±0.17) was significantly greater than all other modalities. CBCT interactively was also more accurate than CBCT statically, and Pan-N was more accurate than Pan-TM and corrected angle linear tomography. CBCT images provide superior reliability and greater accuracy than other modalities in the detection of condylar cortical erosion. The CBCT and spiral CT methods were highly reliable for evaluation of the bony mandibular condyle. In the several cases, the bone abnormalities of the autopsy specimens were found with both methods by carefully analyzing all of the images (Honda et al. 2006). Being less expensive and with considerably lower radiation dose in patient examinations, CBCT is both a cost and a dose-effective alternative diagnostic method for examination of the bony components of the TMJ (Honda et al. 2006).

In this regard, Madani et al. (2015) in a recent study using 34 cases for evaluation of degenerative changes, condylar position and joint effusion in patients with TMD via MRI revealed about the condylar position in the fossa that 32 of the examined joints (47.1%) were in central position, 30 (44.1%) were in posterior position, and 6 joints (8.8%) were in the upper position. Moreover, 34 joints (70.8%) had clicks, and 14 joints (70%) didn't show clicks or symptoms of osteoarthritis. Following that, grade-zero and grade-one effusions accounted for the highest number of examined joints, and there was no significant relationship between effusion volume and type of clicking. 10 cases (14.7%) had premature clicks, 20 (29.4%) had intermediate clicks, and 18 joints (26.5%) had delayed clicks.

Several radiographic methods are used to assess the TMJ, a field difficult to be imaged due to factors like superimposition of adjacent structures and morphological variations. The complexity of the TMD however, demands a clear and precise image of the region for effective management of the patient. CBCT provides a definite advantage over other techniques due to its low radiation dose to patient, smaller equipment and ability to provide multi-planar reformation and 3D images and there are promising researches in the field of CBCT in TMJ imaging (Krishnamoorthy et al. 2013).

In conclusion, the most prevalent bone changes related to TMD included flattening, erosion and osteophyte. The changes were highly reported for TMD than healthy individuals and no significant correlation was found between TMJ bone changes and the patients' age and gender. Similar comparative studies are needed to demonstrate the full spectrum of TMJ articular dysmorphology and determine whether the accuracy of CBCT remains high.

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Adhesion of *Streptococcus mutans* on glazed IPS e.max press, glazed feldspatic and dental enamel

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ABSTRACT

Despite several researches done to determine accuracy of microbial growth in the restoration treatment, the mechanisms for these reports is still unclear.The aim of the study was to comparison adhesion of the *Streptococcus mutans* on glazed IPS e.max press, glazed feldspatic and dental enamel. The in vitro study was done on 15 samples: 5 glazed IPS e.max, 5 glazed feldspatic and 5 dental enamels for vicinity of the bacterial suspension containing *Streptococcus mutans* (10×10^6 cell/mLit). After 48 hours, *Streptococcus mutans* colonies were counted with the naked eye. The mean *Streptococcus mutans* attached to dental enamel was 24.4±8.44 (P<0.001). The *Streptococcus mutans* attached to glazed IPS e.max was 1.8±0.83. The *Streptococcus mutans* attached to glazed feldspatic was 1.4±0.54. No significant differences observed between the IPS e.max and feldspatic (P<0.8). The results showed *Streptococcus mutans* adhesion to enamel was higher than glazed IPS e.max and glazed feldspatic ceramic material.

KEY WORDS: IPS E.MAX, FELDSPATIC, *STREPTOCOCCUS MUTANS*

INTRODUCTION

There is a rich ecosystem in the oral cavity, with a countless number of microorganisms. Although both periodontal disease and dental caries are considered multifactorial diseases, the bacteria in the dental plaque are the main factor in their onset and progression. Increased oral microbiota of *Streptococcus mutans* and *Lactobacillus* is associated with the onset of tooth demineralization and periodontal disease. This condition is much more frequent in orthodontic patients with greater risk of colonization by these microorganisms (Brusca et al. 2007, Harikrishnan et al. 2013, Nascimento et al. 2014 Jalalian et al. 2015Duymus et al. 2016).

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Despite several researches done to determine accuracy of microbial growth in the restoration treatment, the mechanisms for these reports is still unclear However the saliva composition and secretion rate also influence plaque formation (Gameiro et al. 2009).Porcelain has excellent esthetic properties and biocompatibility, and major emphasis in research have been directed toward the enhancement of its strength and aesthetic properties (Rashid, 2014).

Scarce reports exist on bacterial adhesion to porcelain restorations (Kamala and Annapurni, 2006). A previous research stated the best results were obtained through glazing, since it provided a surface topography with minimal bacterial affinity (Sarac et al. 2006). Additionally, it is demonstrated that polished surfaces had lower bacterial adhesion than glazed surfaces (Kawai et al. 2000). In a similar study, Jalalian et al. (2015) reported the adhesion of *Streptococcus mutans* to the enamel was higher than that to polished IPS e.max Press and polished feldspathic porcelain. However, there is no report comparing effect of porcelains with natural dental enamel. So, the aim of the current study was to determine adhesion of the *Streptococcus mutans* on glazed IPS e.max press, glazed feldspatic and dental enamel adhesion using in vitro condition.

MATERIAL AND METHODS

The *in vitro* study was done on 15 samples: 5 glazed IPS e.max, 5 glazed feldspatic and 5 dental enamels for vicinity of the bacterial suspension containing *Streptococcus mutans* (10^9 cell/mLit). The samples had diameter 5× 2mm in laboratory, then phosphate base fabricated. The feldspatic samples fabricated using feldspatic powders (lvocular, Germany) based on manufacture instructions. Ito fabricate IPS e.max press samples, 2 × 5 mm were blocks produced. The dental enamels obtained from normal premolar using diamond disks. The samples glazed at 625°C beginning temperature and increased each 20 minutes until final 920°C, then cooled in fresh air. Samples washed using distilled water then autoclave.

STUDY PROTOCOL

To increase hygiene condition, all samples were located into ultrasonic system for 15 min and then transferred into 70 % alcohol for 30 min. the being sterile of the samples was tested using BHI condition for 24 h. Saliva samples obtained from 2 healthy patients which had no medication for last 3 months without dental caries or periodontal disease. The saliva samples sterile using autoclave (Garcez et al. 2011). Then samples coated with saliva, put into glass vials and immersed in 2mL of *Streptococcus mutans* (PTCCI 683) suspension (×10^9 CFU) and incubated in 37 °C and 5% CO_2 for 24 h. Samples washed 3 times with normal saline, immersed into 2 mL of normal saline and shacked for 2 min (Fournier et al. 1998). Obtained suspension cultured on blood agar and incubated in 37 °C with 5% CO_2 for 48h and the colonies counted (Kantoriski et al. 2006). After 48 hours, *Streptococcus mutans* colonies were counted with the naked eye.

STATISTICAL ANALYSIS

Data for bacteria load was analyzed by one way analysis of variance (ANOVA) using SPSS 16.0 for Windows and is presented as mean ± Sd. For treatments showing a main effect by ANOVA, means were compared using Tukey HSD test. $P<0.05$ was considered as significant differences between treatments.

RESULTS AND DISCUSSION

As seen in table, the mean *Streptococcus mutans* attached to dental enamel was 24.4±8.44 ($P<0.001$). The *Streptococcus mutans* attached to glazed IPS e.max was 1.8±0.83. The *Streptococcus mutans* attached to glazed feldspatic was 1.4±0.54. No significant differences observed between the IPS e.max and feldspatic ($P<0.8$).

Table 1. The adhesion of streptococcus mutans on different restoration

	adhesion	C.V
Feldspatic	1.4±0.54	38.57
IPS e.max	1.8±0.83	46.11
Dental enamel	24.4±8.44	34.59
P value	0.001	

As observed in the current study, *Streptococcus mutans* attached was lower in glazed feldspatic< IPS e.max, dental enamel. However, no significant difference observed between glazed feldspatic and IPS e.max. Bacteria-dental interactions typical of enamel or cementum surfaces, *in vivo* biofilm formation on restorative surfaces have physiochemical and biochemical interactions (Hara and Zero, 2010). Pathogenic communities involving *Bifidobacterium dentium*, *Scardovia wiggsiae*, *Bifidobacterium longum*, *Bifidobacterium adolecentis*, *Prevotella spp*, *Selenomonas spp* and *Lactobacilli spp* have also been demonstrated to be complicit in the etiology of dental caries (Zhang et al. 2015). *Streptococcus mutans* has been demonstrated as the primary etiologic agent in caries initiation and reductionist approach can elucidate vital information regarding its interaction with restoration surfaces; however, additional studies may also use a more holistic approach (Wessel et al. 2014).

The first stage of colonization by an organism involves adherence of the organism to a host surface. From this viewpoint, evaluation of *Streptococcus mutans* adhesion and colonization to tooth surfaces and restorative materials are of most importance for their success (Lassila et al. 2009). Eick et al. (2004) demonstrated that no correlation found between surface roughness and the number of *Streptococcus mutans*. In the oral environment, the adsorption of salivary proteins to the tooth or restorative surface precedes and promotes bacterial adherence. They can form an acquired salivary pellicle to which bacteria and structural substrates may bind (Keulemans et al. 2009). Plaque accumulation was more influenced by the presence of a salivary pellicle than by material type. Viability, however, was influenced by material composition, in this case, differentiated by glass content (Dittmer et al. 2015).

Feldspathic porcelains are usually used as a veneering material for metal ceramic restorations and provide excellent esthetics and compressive strength (Duymus et al. 2016). Otherwise, the rough porcelain surface is prone to adhesion and retention of oral microorganisms causing excessive plaque accumulation, gingival irritation, increased surface staining and poor esthetics of the restored teeth and thereby increasing the risk of dental caries and periodontal disease (Hengtrakool et al. 2011).

The oral cavity is a complex, aqueous environment where the restorative material is in contact with saliva (Hengtrakool et al. 2011). Other factors such as low pH due to acidic foods and drinks may influence the material's mechanical and physical characteristics (Honorio et al. 2008). The availability and long-term success of prosthesis, depends upon the protection of the polished surface. The degradation of surface finish will cause the formation of surface cracks and after a while, leaving the porcelain metal sub-structure. In addition, surface deterioration will facilitate the involvement of plaque and microorganisms (Honorio et al. 2008).

Karayazgan et al. (2010) reported that the level of adhesion of Candida albicans to the polished surface of feldspathic porcelain was 3.4 ± 0.25 colonies/mm^2. In a similar study, enamel used as the control for assessment of the adhesion of *Streptococcus mutans* to uncoated and saliva-coated glass ceramics and composites (Kantorski et al. 2008) and their report was consistent with the findings of the current study. In a research on the adhesion to different the ceramics, composites and amalgam concluded the bacterial affinity was equal in all groups of ceramics assessed (Kawai et al. 2000). In conclusion the results showed *Streptococcus mutans* adhesion to enamel was higher than glazed IPS e.max and glazed feldspatic ceramic material. According to the findings of the present study, polished IPS e.max Press and polished feldspathic porcelain exhibit similar characteristics in terms of bacterial adhesion and either one can be the choice material.

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Comparison of surface roughness between CO_2 laser and typical glazing on two types of porcelain vita and ivoclar

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ABSTRACT

Existence defects in porcelain is directly related to the techniques of porcelain and firing cycles. Adjust glazed surface during clinical work to correct occlusal interferences surface and proximal are common, Which Leads to increased surface roughness. So do Glaze and polishing after Adjust process is essential. In this in vitro study evaluated two types of commercial porcelain with different crystalline content, VITA (Zahnfabrik, Bad Säckingen, Germany) and porcelain IPS d.Sign (Ivoclar Vivadent, Amherst, NY) each of the samples of porcelain were divided into 4 groups according to the two types of porcelain used, generally divided into 8 groups were divided. The laser co2 laser (ultra-dream pluse, Guro-Gu, Seoul, Korea) wavelength 10.6 (mμ) and with the 30W and the time of exposure 1 and 1/5 minutes was used. Standard polished group with sandy paper with a grit similar, According to the America Academy aesthetic dentistry was prepare. Surface roughness were evaluated using a profilometer and the two-way ANOVA test and post hoc Tukey test was used for statistical analysis of the samples. The findings of this study indicated that porcelain surface roughness by CO_2 laser glazed significantly less than typical Glaze, however, ANOVA test between average 5 groups length consecutive depth, did not show significant differences, Also, the surface roughness of CO_2 laser glazed porcelain were significantly lower than the polished porcelain. The surface roughness of glazed porcelain by laser CO_2 was less than of typical glaze.

INTRODUCTION

Dental porcelains have a significant role in dentistry due to properties such as color stability and chemical stability and low thermal conductivity and high bio compatibility but limited fracture strength of these materials limits their application. Presence of available defects in porcelain has a major effect on reducing life span of porcelain. Presence of available defects has direct relationship with process technique and porcelain firing

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cycles. Surface roughness increases followed by porcelain adjustment of surface pore, which acts as an important center of stress focus and often leads to catastrophic failure, (Morena 1986 Scherrer 1999, Griggs 1996, Kelly 1997, Raigrodski 2001 Raigrodski 2004, Gonzaga 2009, Garcia 2015, Alavi 2017 and Lohbauer 2017).

Glazing includes porcelain firingcycle near the sintering temperature (Chang 2011) and (Quinn 2012) porcelain surface melts during this cycle and Glassy Phase fillsSurface irregularities, the glazing is done as auto glaze and over glaze that both of whichglazingtechniques createsmoother surface with higher glass level and lower flows (Eppler 1983) and (Marshall 1993). Papers show that glazing has important role in reducing plaque accumulation in the porcelain surface (Brackett 1989) and (Motro 2012) and (Yilmaz 2010).

Using microwave techniques has been proposed as an alternative to typical glazing recently (Barghi 1976). Microwave leads to less surface defect and a smoother surface and requires lesstime. Using Co_2 laser leads to reducing surface roughness in comparison with typical laser but just in laser with high power co_2, better color has shown than typical glazing (Podshadley 1966). Porcelain surface density is created by different techniques in a wide range of ceramic materials which can be done by Thermal, mechanical and polished firing and putting a layer of porcelain with coefficient thermal expansion less than porcelain. CO_2 laser is very convenient for improving the level of dental porcelain because the emitted wavelength by this laser is absorbed entirely by porcelain (Fairhurst 1992). It was suggested that surface treatment of porcelain by laser prevents formation of micro cracks and leads to increased mechanical strength of porcelain. The present study reviews CO_2 laser as a surface treatment for porcelain glaze and compares results with typical glazing.

MATERIALS AND METHODS

In this study which is empirical_Laboratory type, 2 types of commercial porcelain with different crystalline content of VITA VM13 (Zahnfabrik, BadSäckingen, Germany) and porcelain IPS d.Sign (Ivoclar Vivadent, Amherst, NY) were selected (Barghi 1976) and (Brentel 2011), a brass generator was used to standardize the samples with 5 × 5 × 10 mm. Porcelain was placed in generator after mixing the powder and liquid. Extra humidity was taken by a handkerchief (Kleenex; Kimberly-clark, Neenah, wis) and all samples were placed on wetness (honey comb mesh tray; American dental supply; Allentown pa) andwere sintered in porcelain furnace (program at p 200; ivoclar vivadent) according to manufacturer's instructions as described below;

First, the samples were dried for three minutes, after3 minutes ofstanding onpreheat stage with speed of 83 ° C per minute were reached to temperature of 913 degrees Celsius and were kept at this temperature for 30 seconds.For sample preparation, two firing cycles were performedfor wind porcelain and enamel and one cycle was performed for glazing. Typical cyclewas roughed by a diamond bur

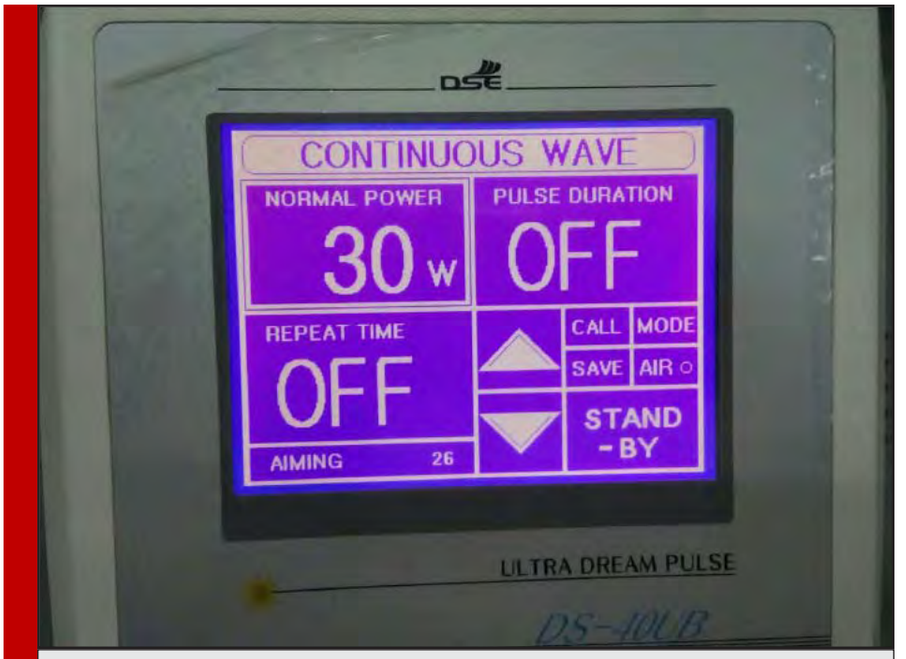

FIGURE 1

FIGURE 2

(2134F, KG Sorensen) with high speed along with water of samples surface (similar to clinical conditions of occlusal adjustment) and were divided into 4 groups that were divided into 8 groups generallybased on porcelain trade type. In this study, co2 laser (ultra-dream pluse, Guro-Gu, Seoul, Korea) was used with a wavelength 10.6 (mμ) and with power 30 W and exposure time 1 and 1/5 minutes. Co2 laser radiation is continuously and focuses directly on a samples that are placed on refractory base. Spot size of co2 laser was 5/0 cm.

Remaining sample of each type of porcelain was glazed in the usual way of furnace glazing and according to glazing manufacturer's instructions, also standard polished group was prepared with sandpaper bysame grits according to recommendation of American Academy of Cosmetic Dentistry (P360, P400 and P1200) (Fairhurst 1992).To investigate surface roughness, a profilometer (Mitutoyo Surf Test 402 Analyser; Mitutoyo crop, Kawasaki, Japan) was used with a pen diameter 12.5 mμ, pen power 10mg (Prasad 2009) and movement speed of co2 laser on the surface was 10 mm /min (Sgura 2015) and manually, and the entire sample length was scanned by profilometer (Mitutoyo Surf Test 402 Analyser; Mitutoyo crop, Kawasaki, Japan).

FIGURE 3

To measure surface roughness of samples by profilometerdevice, porcelains sample was placed in such a way that device indenter placed in middle part of sample in terms of width, then three times measurement were performed for each sample in this way, that average of three measured values for each sample was considered as an average of surface roughness for that sample. Parameters of the surface roughness were calculated by Ra (average of surface roughness heights that is obtained from dividing total area of roughness on sample length) and Rz (a fifth of sum of 5 consecutive peaks of surface roughness or in other words the average of the tallest roughness peaks (heights) in 5 sequence length of the sample that is measured according to micrometer) and Rpm /rz (Rpm is the average of depth length of 5 samples subsequent length). Two-way ANOVA test and post hoc Tukey test was used for statistical analysis of the samples.

DISCUSSION

According to performed statistical tests in this study and obtained data from these tests, VITA porcelains group

FIGURE 4

which had been typically glazed,had no significant difference with any group, but IVOCLAR porcelains group that was typically glazed had more surface roughness compared to VITA porcelains group which had been glazed with laser for 1.5 minutes. Although Vita porcelains that had been typically glazed, had no significant difference with any group, detection probability of this difference was existed in case of using more accurate methods such as SEM.

In particular, use of SEM could provide worthy help in detecting a difference of surface roughness in typical glazing groups with groups that were glazed withCO2 laser. Also, porcelains Microstructure structure can be a reason for mechanical and apparent difference of porcelain, that this matter can be a justification for providing different results, for example, in study of Sarac et al. (2005) difference in samples harness had been expressed as an evidence for providing different results in similar methods of polishing, Also, inmentioned study has been stated that the difference in surface roughness of the samples can be due to differences in samples characteristics (Sarac 2006); therefore, it is advised to evaluate structure of porcelains Microstructure with using X-ray in future studies.

There is significant difference between VITA porcelains group that have been glazed with co2 laser for 1 minute with VITA porcelains group that have been glazed with co2 laser for 1.5 minutes, whichis demonstrator of effect of time of using CO_2 laser for glazing. Regarding the IVOCLAR porcelains, in case of using accurate methods to survey the surface roughness, it was possible that time of using co2 laser had significant difference in surface roughness of samples in mentioned porcelain. Therefore, using more accurate methods of handling surface roughness such as use of SEM is suggested for further study.

Reham & coworkers in 2014 surveyed the roughness and surface properties of porcelain after typical glazing, glazing with xeclexcimer and CO_2 laser. They observedmore smoother and a more uniform surface than typical glazing in the survey with electron microscope in high power of both laser (Reham et al., 2014), which is seconder of present study findings.

ANOVA test also showed that there is significant difference between Rz of used porcelain groups in study that had been glazed typical with groups that had been glazed with co_2 laser (Rzof groups that have been glazed with co_2 laser is lower than groups that have been typically glazed) but significant difference was not observed between Ra and Rpm of groups. Ricardo and co workers considered CO_2 laser with three different powers as an alternative to oven glaze on 2 types of porcelain in 2013. Results of this study showed that the amount of surface roughness had no significant difference among various brands ofporcelain. They observed reduction of surface roughness in laser group with power 45w/cm2 compared to the control group but significant difference was not observed between other groups (Ricardo et al., 2013).

In present study, similar results of this study were obtained and was shown that, there is difference between two types of porcelain (2 brand types) that was used in the study only when glazing method was used by CO_2 laser in terms of surface roughness and otherwise, there is no significant difference between the types of different not glazed (only were polished) porcelain (different brands), it should be noted here that a large number of studies only survey the Ra parameter (Prasad 2009) and (Yilmaz 2010) and (Güler 2009); while due to importance and impact of the Rz parameter, some differences in this study can be justified with other studies to this reason, also this problem is demonstrator of measurement necessity of the Rz parameter to identify surface roughness more accurately. Furthermore, surveying more parameters about surface roughness as was done in this study, can provide more useful information about surface morphology to us, it should be noted that the use of the Rz parameter and ratio Rpm / Rz was essential perfectly alongside Ra parameter and using mentioned parameters in present study is another reason for difference of some findings of this study with other studies and this is the strength of present study. Also, in present study, glazed groups that were only polished had no significant difference.

Byeong-Hoon and his colleagues performed a study in 2013 and surveyed the improvement of feldspathic porcelain surface roughness by a new rubber wheels which contained uniform particles of carbide silicon and small diamond particles on two sinterd and build porcelain in CAD-CAM method. The surface roughness of both porcelains in surface polishing methods was lower than glazing method (Huaw 2013), which reasons the differences between findings of Byung-Hoonand his colleagues with present study can be known in lack of our different polishingmethods usage, using different commercial brands of materials, different used methodologies in the studies and different investigated variables.

In the present study, significant difference was not observed in their surface roughness between different porcelains with same method of glazing, but a significant difference was observed between porcelains that had been glazed with co2 laser method with porcelains that had beentypically glazed or with porcelains that had not been glazed. Sumitsethi and his colleagues surveyed and compared surface roughness inautogaze, reglaze and chair side polished method of porcelain surface in two vita vmk94 andivoclar classic porcelain in 2012. Their results showed that different types of porcelains (different brands) had no significant difference in terms of surface roughness in different glazing methods (Prasad 2009), which is confirmation of present study. As mentioned in present study, no significant difference was not observed between control groups that had been only polished in terms of surface roughness, but it was possible toobserve a similar result to study of Vieira et al, where different polishing methods were used (Vieira et al., 2013), because they observed that the lowest surface roughness is related to VM7 ceramics with polishing method with Shofu system between VM7 and VM13 ceramics that had been studied, in the study thatevaluate and surveythesurface roughness in different ways offinishing and polishing dental ceramics that also had significant difference with other groups and other methods of finishing and polishing statistically.

According to a study of Barghi and associates (1975) and study of Karaksi and associates (1993), the best mode of surface roughness that leads to smoother surface and increasedmechanical strength of the material Compared with different systems of polishing and finishing of using glazing (Barghi 1975) and (El-Karaksi 1993), that their findings areconfirmation of the results of present study based onexistenceof a significant difference between the glazed groups with not glazed groups. Fuzzi and coworkers (Fuzzi et al., 1996) also in a study that performed about evaluating surface roughness in different ways of polishing and glazing in 1996, concluded that glazing method is preferable to polishing method which their findings are confirmation of the results of present study. Also Brackett SE et al. (1989) preferred glazing method in a study that examined the effect of glazing method in porcelain strength and surface modifications (Brackett 1989). Also Al-Wahadni (2006) observed in his study that glazing causes creating a smoother surface and less surface roughness compared to different methods of finishing and polishing (Al-Wahadni 2006), although present study and many other studies are confirmation of these findings based on priority of glazing in reducing the surface roughness compared with different methods of polishing and finishing.

However, some studies, including Kelly and colleagues (1996) were presented different results, theydidn't observe significant difference about surface roughness in their study between glazing method with finishing and polishing in different ceramics (Kelly et al. 1996); demonstrated that results of these findings can be found in type of used ceramics and used polishing, finishing and glazing methods instudy and used laboratory equipment. In contrast to our findings, Sarac and coworkers (2006) demonstrated that there is no significant statistical difference between surface roughness of glazed ceramicswith polished ceramic with different systems of polishing, Furthermore, there are studies that have been claimed that the surface roughness of polished ceramicsare less than glazed ceramics, like the Hulterstro¨m et al. (1993) and NettoJu'nior et al. (2006) and Wright (2004); (Hulterström 1993) and (Wright 2004), but the findings difference can be found in used ceramics, exist-

ence of laboratory errors and used methods in glazing and polishing materials.

Also, we can claim based on mentioned studies that consensus is not available between researchers about the effects of glazing or different ways of finishing and polishing to reduce the surface roughness of ceramic and porcelains, So, existence of different and even contradictory findings in studies is not far, results can be different due to use of produced commercial different brands, different used methodologies in the studies and different investigated variables and way of porcelain preparation, for example, the temperature at which the samples are prepared in their presence can have big impact in surface roughness of samples,we should note that created temperature by thermocouple can't alwaysbe demonstrator ofreal temperature, because the surrounding temperature can cause the loss of some of these temperatures. If you have not considered this matter, it may provide different and even conflicting results about the surface roughness in different glazing methods.

Sarikaya and Güler (2010) addressed to effect of different polishing techniques at the surfaces ofdental porcelains surface roughness in a study. Their findings which is confirmation ofpresent study findings also wad showed that using glazing can reduce the surface roughness compared to other polishing methods (Sarikaya 2010). Also, Tholt et al. (2006) surveyed and measured the surface roughness of prepared dental ceramics with different finishing techniques by using Atomic Force Microscope and Profilometer, (Tholt et al., 2006). They found that there are differences betweendifferent porcelains with different ways of polishing, as well as they found as present study that glazing method causes less surface roughness compared with other finishing and polishing methods. Speed of the used method in present study for porcelain glazing, is the main advantage of this method compared to autoglazing techniques to typical method.

CONCLUSION

RZ item of porcelains surface roughness amount (Average of maximum violence height)with typical glazingwas significantly greatercompared to porcelains that were glazed by co_2 laser, although the ANOVA test between Ra, Rpm groups didn't show significant difference. Also, not glazed porcelains that had just polished, had more surface roughness significantly compared to porcelains that were glazed by co_2 laser.

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Comparative studies on cooking qualities of aged brown and polished rice, *Oryza sativa*

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ABSTRACT

A comparative study of cooking quality of brown rice and polished rice (i.e. volume, length, breadth, thickness expansion ratio, water uptake ratio and optimum cooking time) with respect to different time was carried out in the laboratory of Post-Harvest Process and Food Engineering College of Agricultural Engineering, JNKVV, Jabalpur. Brown rice and polished rice was procured from Singhai Rice Mill village Panagar, Jabalpur and kept for three months at ambient condition. Sample from aged raw and polished rice were withdrawn and cooked for 3, 5 and 7 minutes for evaluation of cooking quality. It was observed that the length expansion ratio (LER) of polished rice was maximum (1.46) as compared to brown rice for all type of cooking, breadth expansion ratio was maximum (1.45), thickness expansion ratio (TER-1.22), volume expansion ratio of polished rice (VER-2.6), the water uptake of polished rice was maximum (1.78) as compared to brown rice. However cooking time was minimum for polished rice when cooked for 3, 5 and 7 minutes as compared to brown rice.

KEY WORDS: BROWN RICE, POLISHED RICE LENGTH, BREADTH, VOLUME EXPANSION RATIO, WATER UPTAKE RATIO, OPTIMUM COOKING TIME

INTRODUCTION

Rice (*Oryza sativa L)* is a cereal grain and an important staple food for a large part of the world's human population. Among cereals, rice is even more nutritious than wheat. India is the world's largest producer of rice. India produced 105 million tonnes rice in the year 2017 (www.fci.gov.in). Brown rice is unpolished whole rice grain that is produced by removing only the husk using the rubber rolls. Distinguish factor is its unpolished features and not the colour. Post-harvest researchers reported that the milling recovery in brown rice is 10% higher than polished rice. It contain minerals (prevents beriberi) and high fat (energy source). Also it has been reported that brown rice contains high phytic acid (antioxidant, anti-cancer); it decreases serum cholesterol (prevents

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cardio-vascular diseases) and is considered a low glycemic index food (low starch, high complex carbohydrates which decrease risk to type 2 diabetes). Moreover, brown rice contains large amount of insoluble fibre, which may prevent a variety of cancers. There are many benefits of brown rice. The fuel savings in milling process is 50-60% because the polishing and whitening steps are eliminated. In this way the milling time is also shortened with less labour requirements, also, the enhancement in output volume and the economy in milling indicated the business opportunity in brown rice (Rogelio, 2003 Kumar et al., 2017).

Brown rice when being cooked, becomes a sticky or pasty mass, swells only slightly and leaves a thick gruel. All these cooking properties may not be accustomed to most consumers who prefer fluffy and hard texture. The desired cooking properties can normally be obtained by storing freshly-harvested rice for at least 3-6 months (Swamy et al. 1978). Ageing involves changes in physical and chemical properties of the rice grain. Starch, protein and lipids are the main grain components which affect cooking and eating quality (Villareal et al.1976). An unusual property of rice is that its cooking quality depends on its age after harvest. New rice swells poorly during cooking. This undesirable property gradually changes during storage for few months (Patindol, et al. 2005 and Sowbhagya 2001).

MATERIALS & METHODS

Brown rice and polished rice were procured from modern rice mill. The rice was stored at ambient condition for four month in the laboratory Post Harvest Process and Food Engineering Department, College of Agricultural Engineering, JNKVV, Jabalpur. Then the samples were withdrawn from the stored rice stock and cooked in the laboratory for different time to evaluate the cooking quality including optimum cooking time.

MOISTURE CONTENT DETERMINATION

Moisture content of sample was determined by standard air oven method (AOAC method). The samples were dried in hot air oven maintained at 103±1°C for 24 hours.

$$Moisture\%\ (w.b.) = \frac{Loss\ in\ weight\ of\ sample}{Initial\ weight\ of\ sample}$$

COOKING QUALITY

Rice expands length wise, breadth wise and thickness wise during cooking by absorbing water. To determine rice length after cooking and rice breadth after cooking 20 milled grains are pre-soaked to 10-30 minute and placed directly into boiling water by directly dropping until its optimum cooking time .The length, breadth and thickness are measured and the average worked out. The experiment was aimed to determine cooking properties. Rice is considered to have good cooking quality if it has high length expansion ratio (VER), volume expansion ratio (LER), water uptake ratio (WUR) and minimum Cooking time (CT) (Juliano, 1985). Length and volume expansion ratio (VER) and water absorption are desirable for the food service industry as they lead to a fuller plate for the same amount of rice. Length expansion ratio i.e. the ratio of the length of cooked rice to that of raw rice was calculated

$$Length\ expansion\ ratio(LER) = \frac{Length\ of\ cooked\ rice}{Length\ of\ raw\ rice}$$

Breadth expansion ratio i.e. the ratio of the breadth of cooked rice to that of raw rice was calculated

$$Breadth\ expansionratio(BER) = \frac{Breadth\ of\ cooked\ rice}{Breadth\ of\ raw\ rice}$$

Thickness expansion ratio i.e. the ratio of the thickness of cooked rice to that of raw rice was calculated

$$Thickness\ expansion\ ratio\ (TER) = \frac{Thickness\ of\ cooked\ rice}{Thickness\ of\ raw\ rice}$$

VOLUME EXPANSION RATIO

Volume expansion ratio (VER) was calculated i.e. the ratio of the volume of the cooked rice to the initial volume of the raw rice, using toluene displacement method.

$$Volume\ expansion\ ratio\ (VER) = \frac{Volume\ of\ the\ cooked\ rice}{volume\ of\ raw\ rice}$$

WATER UPTAKE RATIO

Water uptake ratio (WUR) as ratio of water absorbed during cooking to cooked rice weight was calculated by weight of cooked rice and weight raw rice.

$$Water\ uptake\ ratio\ (WUR) = \frac{Weight\ of\ cooked\ rice}{Weight\ of\ raw\ rice}$$

OPTIMUM COOKING TIME (OCT)

It is the time taken by a predetermined weight of rice to cook completely. Brown rice and polished rice (2g) samples were taken in test tube and cooked in 100ml distilled water in a hot water bath at 100°C. The cooking time was determined by taking out 20 grains at different time intervals during cooking and pressing them between two glass plates until atleast 90% of rice had no longer centres, which is considered as optimum cooking time.

RESULTS AND DISCUSSION

A comparative study of cooking quality of brown rice and polished rice was initiated to find out significance of polishing of rice. Three aged brown and polished rice was cooked for three minute and 1.135 times increases in length were observed with linear expansion. The graph reveals about the forecast value of length expansion of polished rice at the time of cooking. The linear relation line will always provide the best result for the relation between time of cooking and expansion of polished rice. The maximum value of R observed as 97% and this variability was explained in the expansion of polished rice through the cooking time.It shows that the expansion of polished rice is certainly dependent on the cooking time means the cooking time is one of the best factor. The length expansion ratio for polished and brown rice is represented by models

$$Y = 0.0677x + 0.98 \quad (1)$$

$$Y = 0.0286x + 0.99 \quad (2)$$

The results of breadth expansion of polished rice (for 5 minute cooking) showed 1.42 times increase as shown in fig. 2. It is observed that the polished rice expand at faster rate. The maximum value of R observed as 99% and this variability was explained in the expansion of polished rice through the cooking time and the breadth expansion ratio for polished rice and brown rice are represented by models

$$Y = 0.0781x + 1.0013 \quad (3)$$

$$Y = 0.054x + 0.9862 \quad (4)$$

The graph shows thickness expansion of polished rice increases at faster rate for 7 minute cooking time with 1.22 times increase in thickness. The linear behaviour is shown in fig. 3. The maximum value of Robserved as 99% and this variability was explained in the thickness expansion of polished rice through cooking time. The thickness expansion ratio for polished rice and brown rice are represented by models.

$$Y = 0.0318x + 0.9985 \quad (5)$$

$$Y = 0.0221x + 0.9638 \quad (6)$$

The volume expansion of polished rice was higher (for 3 minute cooking time) with1.8 times increase in volume as shown in fig. 4. It is observed that the polished rice expand at faster rate. The maximum value Robserved as 99% and this variability was explained in the expansion of polished rice through the cooking time. The expansion behaviour can be expressed by following models:

$$Y = 0.257x + 1.0112 \quad (7)$$

$$Y = 0.1234x + 0.9989 \quad (8)$$

The water uptake ratio increased linearly for polished rice (for 5 minute cooking time) with 1.58 times increase as shown in fig. 5. The maximum value of R observed as 99% and this variability was explained in the expansion of polished rice and the water uptake ratio of polished and brown rice behaviour can be shown by following models

$$Y = 0.1066x + 1.0225 \quad (9)$$

$$Y = 0.0916x + 0.9865 \quad (10)$$

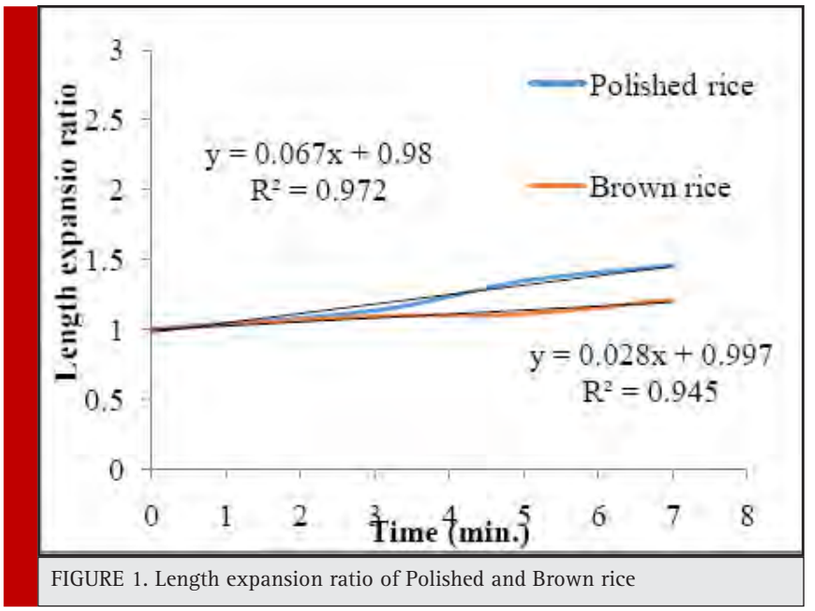

FIGURE 1. Length expansion ratio of Polished and Brown rice

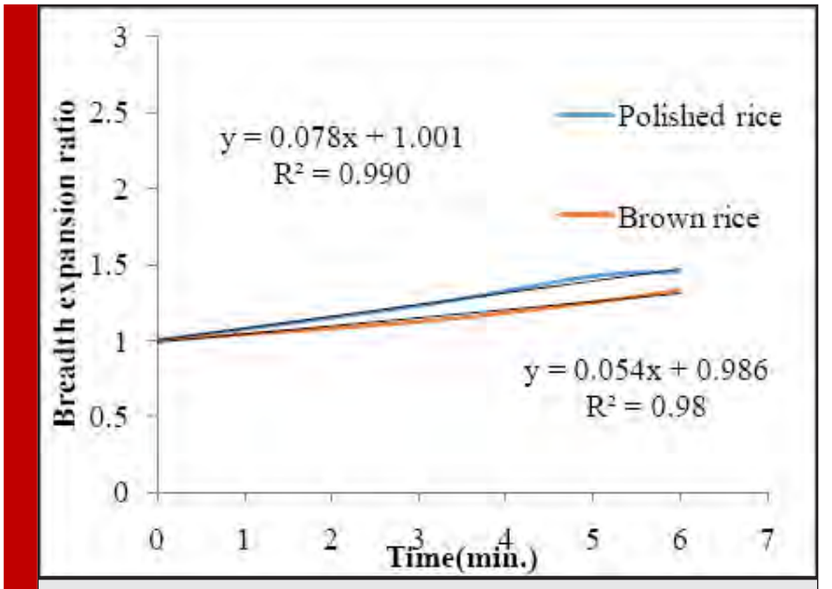

FIGURE 2. Breadth expansion ratio of Polished and Brown rice

Less expansion is observed in brown rice in all three dimensions (length. breadth and thickness) because the kernel is covered with thin layer of bran which contains oil so that expansion ratio was less in brown rice, Although the brown rice takes more time in cooking but water uptake ratio is high in polished rice. In this study length expansion ratio, width expansion ratio, thickness expansion ratio, volume expansion ratio and water uptake ratio of brown and polished rice were determined. Rice is considered to have good cooking quality if it has high expansion ratio. Water to rice ratio affected chewiness of cooked rice. Ratio of water to rice also affected the eating quality rice (Daomukda 2011).

Length expansion ratio of brown and polished rice is shown in figure 1. Cooking time of rice varied from 3-7 minutes (with 2 min interval). Polished rice had highest LER as compared to brown rice. This shows the positive correlation of LER with time of cooking (Gujral 2003).

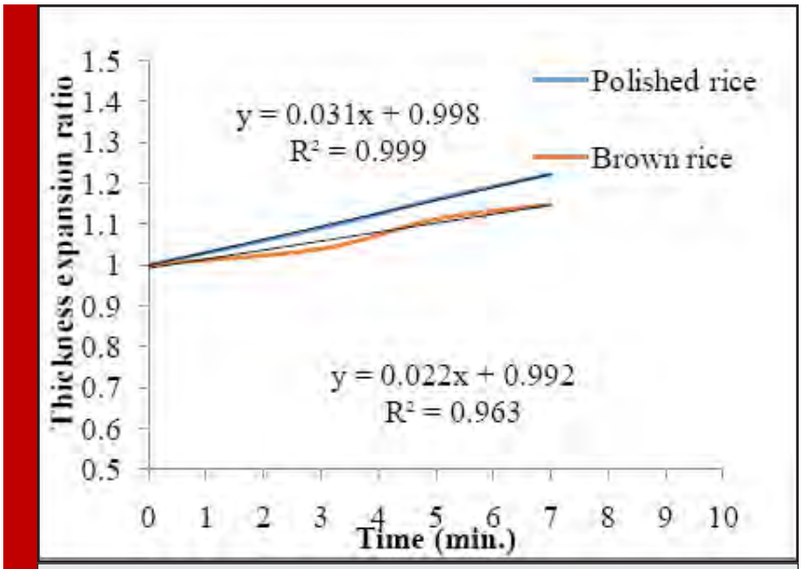

FIGURE 3. Thickness expansion ratio of Polished and Brown rice

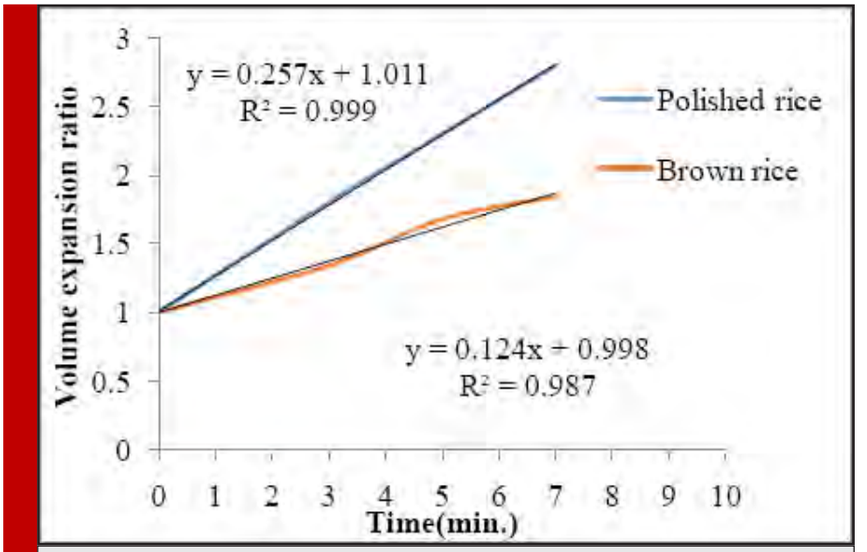

FIGURE 4. Volume expansion ratio of Polished and Brown rice

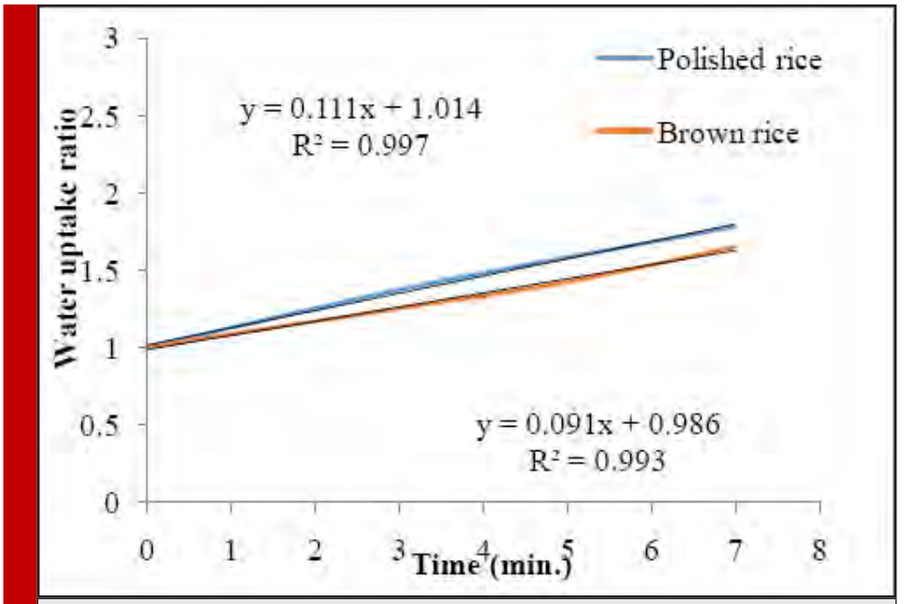

FIGURE 5. Water uptake ratio expansion of Polished and Brown rice

Similar results of BER, VER and WUR were also obtained in brown and polished rice. A greater elongation ratio of polished rice is due to absence of bran layer which allows water to enter inside the kernel as compared to brown rice. The results are in agreement with Kumar et al. (2017).

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Perceived challenges by the Iranian baccalaureate surgical technology students in their clinical education: A qualitative study

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ABSTRACT

The operating room field in Iran is a branch of medical sciences during this period, students became become familiar with the new principles of the operation room and modern surgical techniques in specialized and super specialized surgeries and they train the care and assistance to the patient's administration before, during and after operation. The purpose of this qualitative study is to investigate how the students are taught, the effective factors and possible challenges related to educational issues in the operating room. This is a qualitative content analysis which was performed in 2014-2015. The participants consisted of 35 beneficiaries in the operating room field, namely, students of different academic terms, matrons, educators (instructors), surgeons and operating room personnel in different academic centers in Khorasan Razavi province who were rich in information and had a tendency to comment on their experience. In this study, sampling was first carried out in a targeted way and then was continued with theoretical sampling and sampling was continued until data saturation. All interviews were recorded and then handwritten and analyzed using continuous comparative method and qualitative content analysis. The profound and deep descriptions of the participants led to the emergence of 13 subcategories and 3 main categories of the "unconventional educational atmosphere", "the challenge of student admission to the surgical team," the "operating room culture" and, finally,

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one main theme of the "dynamic nature of the operating room".The special educational environment in the operating room environment, lack of acceptance of students in the surgical team as a training member and hierarchical culture of the operating room, has created problems for the training of these students. In fact, the clinical training of operating room students is based on teamwork and the members of the surgical team are, in some cases, instructors in the shade. Given the dynamic nature of the operating room, collaboration and participation between educational and clinical institutions can help improve the learning environment, acceptance students in the surgical team as a training member, and creating a dynamic, collaborative and flexible culture.

KEY WORDS: PERCEIVED CHALLENGES IRANIAN BACCALAUREATE SURGICAL TECHNOLOGY STUDENTS

INTRODUCTION

Bachelor of Science in surgical technology in Iran is a branch of medical science during which the students become familiar with new principles of surgical technologies in specialty and subspecialty surgeries and they learn how to care and help the patients before, during and after surgery (Operating Room and Anesthesia,2017). Training programs of operating rooms are offered in two ways worldwide: in the first case, nursing graduates (after achieving a bachelor's degree) are trained in a period of 1-2 years to enter the operating room. In the second case, surgical technicians, after general training of the operating room, are prepared to act as a mobile and scrubs and then, if desired, they are prepared as the first help of the surgeons in a period of 1/5 to 2/5 years. Standards of patient care in the community of operating room nurses in America also show that students of operating room technician can pursue their education in specialized courses in higher education. The people of each period have their own duties and the role of these specialized people is different from the operating room technician. Operating room is a complex system that coordinates the individual, technology, and patients in a physical environment to achieve favorable outcomes in patients (Islamic Republic of Iran Ministry of Health and Treatment and Medical Education Supreme Council for Planning of Medical Sciences, 2007). Operating room is considered as high-risk environment for patients, because in spite of the small number of cases, some potential problems of patients after the surgery are due to the mistakes of the operating room personnel that can lead to the death of the patients (Deyoung 2009).

In Iran, students of surgical technology are admitted directly based on the exam and with no relation to nursing. This field, in different colleges between the years 1987 to 2001 and it was launched at the undergraduate across the country, was launched in the associate degree level in 2009 (Operating Room and Anesthesia, 2017). Due to the fact that now in Iran there is no training courses in doctoral degree (Ph.D.) for surgical technology and its MA was launched only two years ago, MS nurses in internal injuries (with work experience in the operating room or an associate degree in surgical technology) take the responsibility of the practical and theoretical training of this group of students in educational centers. Given that the field is new and considering the volume increasing of training centers for students, it is not considered the required infrastructures such as specialist human resources in clinical training and educational facilities in proportion to the number of students. There is not enough number of teachers in the field, so in many centers teaching this group, traditionally, is given to the supervisors and operating room personnel. Subsequently, due to lack of necessary knowledge of educational affairs, students have faced with problems in their training (Bahrami et al 2014).

The purpose of the training is the optimal and effective care of patients before, during and after the surgery especially in the specialized surgeries, control and prevention of nosocomial infections as well as creating the perfect mental conditions for patients and proper maintenance of devices and medical equipment (Islamic Republic of Iran Ministry of Health and Treatment and Medical Education Supreme Council for Planning of Medical Sciences 2007). However, some items such as technical approach to the field, high expectation of the graduates in this field to help surgeons, and separation from nursing have caused the graduates to not have enough knowledge of nursing care of patients before and after surgery as well as communication with patients. So, they have just learned the technical skills during the surgery (Bailey 2010).

Foreign countries' researches have reported about training in the operating room which is connected with the collaboration (Silen-Lipponen et al., 2004) stress, communication failures (Laur et al., 2012), the effect of stress on other members and students (Kenton 2006) and to the development of the group of Skoczyl et al. (2005). The difference in the education of graduates of the surgical technology in Iran and other countries has caused the results of the studies in the field to be inapplicable in the country. Unfortunately, there is not extensive and efficient research in the surgical technology in the country. Conducted researches are quantitative and investigate certain aspects of education in surgical study. So,

they are not able to analyze all aspects of education in the surgical technology (Bahrami et al., 2014; Khazaei et al., 2014, Tazakori et al., 2016).

However, qualitative approaches have holistic approach to the phenomenon and gain precious information about the training in the clinical environment. The aim of this qualitative content analysis is the evaluation of teaching the students, their required skills, effective factors and potential challenges associated with educational issues in the operating room. Planning to eliminate the defects of the field improves the knowledge and skills of students in this field, provides better care for patients, and ultimately improves the health system.

MATERIAL AND METHODS

The study is a qualitative content analysis study (part of a larger study). It was conducted in 2014-2016 to reveal the problems and challenges of education in the field of surgical technology in Iran. The environment includes all the operating rooms of educational hospitals of Khorasan Razavi (Mashhad, Nishabur, Torbat Heidarieh and Gonabad). The interview was done in places that are accessible to participants or places they desired. The participants were 35 of beneficiaries of education in surgical technology such as the students of different academic semesters, head nurses, teachers, surgeons and operating room personnel at various universities in Khorasan Razavi province. They have precious information and they were willing to recount their experiences. Sampling was done by using purposive sampling and continued by theoretical sampling. Some of the characteristics of the participants are as follows: willingness to participate in research and express their experiences, students who had spent at least one training courses, educators who had at least a year of training in the operating room, and operating room personnel who had at least one year of work experience in the room.

Data were collected by using semi-structured individual interviews. The interview was started by general questions regarding the interview directory and was guided by the responses of participants. After explaining the objectives of the plan to participants and obtaining informed consent, data collection was began. The main question were as follows: what makes you to call a day of the training as good and useful? Or feel the training is not of enough interest today? Mention a good experience and a bad experience from your training in the operating room? What are the facilitating and impeding factors in your clinical training?

A total of 37 interviews was carried out (35 initial interview and two follow-up interviews). The study continued from Tir 93 to Azar 95 (Iranian year). The researchers tried to consider the maximum variation in the sampling; (14 students, 6 Operation room personnel with associated degree or BS in surgical technology or BS in nursing, 4 operating room heads, 6 training teacher, one Dean of the Faculty, 3 surgical assistants and one surgical staff). Educational centers of Khorasan Razavi included Mashhad, Torbat Heydarieh, Gonabad and Nishabur. Data were analyzed according to conventional content analysis.

The data analysis was based on Graneheim and Lundman in seven steps (Graneheim and Lundman 2004). In the first step, all interviews were recorded and transcribed. Then, the decision made about the analysis unit and before starting the coding process, text of interviews was read several times as the unit of analysis. Meaning Units were also read and reviewed several times and unrelated statements were deleted. First, some units were defined and relevant codes of each Meaning unit were written and then the codes were classified inductively and based on common sense and meaning.

The process of data reduction was continued in all units of analysis and primary and secondary classes. In other words, subclasses with similar events are grouped together as classes and then primary classes formed and this trend continued up to the theme. It should be noted that during the analysis the necessary changes were done in the name of the classes which must show the content of the class. The analysis was continually conducted by adding up each interview and the classes were modified. To simplify the process of data analysis,

Work experience in the operation room	Gender	Age	Number	Participants
Semester 4 to 8	11 females/3 males	22-20	14	Student
3-20 years	5 females/1 male	48-35	6	Training teacher
8-25 years	5 females/1 male	55-32	6	Operating room personnel
-	Male	52	1	Dean of the Faculty
4-5 years	Chanbge this year to English Calender Male	48-32	3	Assistant
17 years	Male	53	1	Staff
15- 29 years	Male	55-32	4	Operating room head

coding, cataloging and classification, comparing various data frequently and retrieving quotes, we used software MAXQDA 10 (Mayring 2000).

To increase the reliability and validity of the results, we used the methods used by Cuba and Lincoln that were quoted by Polit and Hungler. The two researchers consider the four criteria of confirm ability, credibility, dependability and transferability as necessary for the consistency and strength of qualitative data (Burn and Grove 2007). Therefore, researchers have helped to validate data with continuous involvement with data, data verification by the participants, allocation of sufficient time to the study and open communication and empathy with the participants. In order to determine the dependability, two separate research teams coded the Interviews that there was a high degree of agreement (85%) between the comments. Also, great care was done in the collection, implementation and recording of data and allocating of enough time to collect the data. As for the transferability, manuscripts, interviews and units of analysis with extracted initial codes were provided to participants and the necessary corrections made and the proposed points were considered. In addition, three expert in the field of qualitative research monitored the study. Using a combination of methods (interviews and field notes) as well as sampling with maximum diversity (interviews with students of different academic semesters from different training centers, faculty-member educators, non-faculty member educators, and surgeons with different expertise) have increased the confirm ability and transferability of the data. In addition, describing the studied background, providing necessary explanations about the participants and using of their direct quotations was also done in this regard.

Ethical considerations in this study include: obtaining ethical permits from Committee for Medical Research Ethics of Medical Sciences of Mashhad (ethical code 940548), explaining the purpose of the study and the use of tape recorder, explaining the way of collecting data for the participants and satisfying them to cooperate, obtaining consent in writing for conscious participation and paying attention to the basics including the research explanation, identifying the purpose of the study, stating the criteria for sample selection, the benefits of participation in the research, ensuring the anonymity, answering questions, the right to refuse to continue the research, providing adequate information and assuring the participants to put the interviews in a safe place.

RESULTS AND DISCUSSION

Data analysis of participants' statements and notes in the field lead to the emergence of 12 subcategories and 3 main categories and eventually one main theme.

Main category: unconventional educational atmosphere

This category includes the following subcategories: lack of facilities and trainers, lack of balance between trainings and goals, red lines, safety and sterility principals, the confrontation of the caring and technical roles of students and the simultaneous presence of other students.

Lack of facilities and teachers: learning environment has been considered as a key environment for education and training of students and for helping to link theory with the practice in the clinical setting. The data in this study showed that the participants are not satisfied with their learning environment. A training teacher with several years of experience in the operating room, says: "In the classroom sometimes it is required to explain or draw a part of something and there is no whiteboard and facilities there. There is no place for equipment and

Main theme	Main category	Subcategory
The dynamic nature of operating room	Unconventional educational environment	Lack of facilities and teachers
		Lack of balance between training and goals
		Red lines, safety and sterility principals
		The confrontation of caring and technical roles of students
		The simultaneous presence of other students
	Challenge to admit students to the surgical team	Annoying students and reduction of team speed
		Lack of required knowledge and skills
		The absence of the trainer and humiliation of the students
	Operating room culture	Teamwork
		Gender discrimination
		Hierarchical structure (physicians governorship)
		Paradox of Professional interactions and inappropriate interactions
		Duality of behaviors and the absence of legalism

relaxation of students and it seems as though they do not have facilities for us". The conversion of associate degree to bachelor and the increase of the number of students regardless of the required infrastructure have created problems. The operating room head who has been in charge of training courses says: "The teacher cannot afford to train the students due to the increasing number of students as well as the presence of students from semester 4, 6, 8,". A student says:" teachers cannot teach us everything and do not have time to do so".In educational centers of cities other than Tehran, students actually cannot see thoracic and cardiac surgeries, advanced neurological and eye surgery. "Due to the low number of operating rooms and lack of variety in surgeries, the trainings in the cities are the same and students see the same surgeries in the semester 8 that had seen in semester 2"

Lack of trainers: it is hard to understand the routine and special procedures of hospitals for educators who temporarily go to the hospital with students. Absence of the trainer or the use of non-professional teachers or staff have affected the clinical training of surgical technology students. A student says: "When we do not have any trainers, the only thing is the attendance. Then, they told us to go to the rooms and it is not important to learn or not to learn". In multiple studies, students' confusion and uncertainty in the absence of the trainer has been reported as factors causing problems.

"Of course, depending on the hospital, when we do not have trainer and the nurse is responsible for checking, there is no problem if we are late. I do not remember any washing. Because we do not have fixed trainer, they do not have us to do morning washing.

Mismatch between educational objectives and training objectives: Data analysis showed that the students complain about the aimless trainings and they consider the lack of transparency in goals and evaluating the training as a major problem. A senior student says: the lesson plan is originally for apprenticeship and they should it to students to understand what they do. In fact, I mean that we should be given a sheet based on which they evaluate us. I have not seen anything and most professors evaluate us based on scrub "Students considered as useless some training courses including recovery or management trainings. A student says: "We have weaknesses in file writing and recording of HIS and I personally cannot do this. At least they should include this in training courses. "A student told about the lack of training in Laparoscopy: "I went to the room for laparoscopic surgery and it was cholecystectomy. I did not know anything about laparoscopy even the theory. The personnel came and taught me quickly.

Red lines, safety and sterility principals: the operating room is a complex system in which an individual coordinates the technology and patients in a physical environment to achieve optimal outcomes in patients. Data analysis showed that the majority of operating room personnel are sensitive to safety and sterility principals of patients. A personnel says:

"If we told them to cover your hair, they would think it is because of Hijab but it is not and if the hair go to the sterile field, it can cause infection and problems for patients. Although we notify them, they hate. The health is important here and you can be as you will out of here. And you have to comply with the rules and standards here." On the other hand, surgeons also have their own reasons for patient safety: "The patient has trusted the surgeon about his life and the decision maker is the surgery team. Survival is not interchangeable with anything. It is not acceptable to put the life of the patient in danger."

Confrontation of caring roles and technical roles of students: in the operating room environment, compared to the other sectors, a specific aspect of care is mostly emphasized that is technical. One of the participants says:"If we look at it as a scientific discipline, we must go beyond the skills and consider it as theory-based thing. The problem here is that it seems most of the duties designated for the graduates of this field is practical, but this field is subdivision of the Nursing Science. The thing that now is happening is the technical look at the graduates of the field. So, they are expected to know the equipment and to help the surgeons in the surgery.

Simultaneous presence of students from other disciplines: the difference in the type of training and simultaneous activity of surgical technology and other students and operating team make the operating room training more different and difficult than other parts of the hospital. Faculty member trainer with 14 years of experience in training in the operating room says: "Simultaneous presence of students with different levels of training from freshmen to residents and even the professor himself in a place leads to different clinical experience in the place and the students cannot keep up with the situation and it is difficult to them.

Second category: the challenge of admitting the students in the surgical team

This category includes annoying students and the reduction of the team speed, lack of the required knowledge and skills of students, the absence of the trainer and the humiliation of students. Data analysis showed that the surgeon believes in the priority of patient safety and the life rather than the training and he/she seeks a surgery with minimal side effects for the patient.

Annoying students and the reduction of the team speed: an operating room personnel in this regard says:"The students are beginners and annoying. The

personnel's works are rapid and easy, but it is not the same for students. They stand there without doing anything even when the assistant wants to move to the other place. Another trainer says:"Students in the operating room are uninvited guests and physicians and surgeons are sensitive to the students. Unfortunately, I'm concerned about the students and I permanently turn around in their room and it leads to sore feet because there is no place to sit.

Lack of required knowledge and skills of students: Interview with operating room assistants showed that admitting the students in the surgery team causes some problems."We had a student that knew no equipment. In the middle of the surgery we had to change him because it was a two hour surgery and we were just cutting the area without doing anything. He had lowered our speed and we had to change him.

Absence of the trainer and the humiliation of students: a student says:

"When we did not have the trainer, the personnel took the responsibility and the only important thing was the attendance. Then we were told to go to the rooms and it was up to us to learn or not to learn. "I thought that I am a useless creature that gets humiliated and the students were blamed for any mistakes."

Or another student talks about the impact of every single person on the admission of the student in the team: "The surgery began and we wanted to go to the room, but the surgeon did not allow. As I said it depends on the surgeon and other factors and all of these are effective in our training.

Third category: The operating room culture

This category includes teamwork, gender discrimination, hierarchical structure (surgeon as the governor), paradox of professional interactions and inappropriate interactions, behavior duality and lack of rule of law.

Teamwork: Since the operating room environment is of interactive and dynamic nature, to make the surgery team more cooperative and to eliminate the confusion of the students in the room, the clinical education program of the students should be transparent for the operating room personnel. Some operating room personnel do not consider the participation in the training as a part of their duties and even disappoint the students and the trainers with inappropriate behavior and create an unfavorable atmosphere. However, some staff play the role of the trainer for the students. "Most guys say that the educator plays an important role in the training. However, I say some personnel are the trainers of the students and they explain for you the techniques step by step in the operating room. Faculty member instructor with 14 years of work experience in training says: "See! It is a teamwork and each and every member of the operating room can have clinical effect. Perhaps the least role is for the head nurse and the personnel in the room have the greatest role in training students.

Gender discrimination: One of the known causes of stress in operating room environment for students is the others' behavior."A senior student says: unfortunately, some personnel or male surgeons work with female students. I like justice not discrimination. It is not fair to dismiss the female students earlier than males. If the female students cry, the personnel and the surgeon will change.

Hierarchical structure (doctor as the governor) researchers believe: a good clinical setting has non-hierarchical structure in which the works are done in teams or groups and there is good communication. A trainer says:"Look! When you have a pretentious assistant who does not know his work well and says I'm very good and am in a hurry, he does not let the students use the equipment correctly. He watches the student carefully. However, a patient assistant says take your time we are not in hurry and even tells anesthesiologist that we need more time. Unfortunately, we don't have many of this surgeons and you have to have a chance to work with them.

Paradox of professional interactions and inappropriate interactions: To make a team work so efficient, team members should be familiar with their capabilities. This leads to appropriate interactions between them and coordinates them. The trainer says:"Even students work more with good communicators. He might not be well in the knowledge, but he is good at communication. When he graduates, he is better than a good student with weak relationship and this is important. One of the operating room personnel in connection with the close interaction in the environment of operating rooms says: "some students abuse this open communication and some of them do not have the capacity of such communication.

Studies have shown that in a good clinical setting the interactions are not top-down and the work is done interactively. Data analysis and statements from students and educators in this study shows that surgeons have a top-down look to surgery team especially surgical technology students. This behavior is not in the framework of the operating room rules."Unfortunately, in Iran the higher the education, the higher the immorality and higher education leads to higher immorality.

Behavior duality and lack of rule of law: in the status quo operating room personnel do not consider the cooperation in training as their duty. And they do not have necessary cooperation with the trainer for providing the required facilities to students and transferring their experiences in the absence of the trainer."The student is of no position and is blamed for everything wrong even it is the fault of the personnel. Another trainer about the ignorance of some surgeons says:"According to the

rules of the school we took a male student to the women operating room. The gynecologist of the room began to quarrel and said to force the student out. Another trainer in connection with the operation room atmosphere says:"There is no law here. If there is a law, they can be persecuted. However, doctors are the governors and we cannot blame them.

Results of the study show that the challenges of clinical education in the operating room in Iran, due to the complex and dynamic nature of operating room, are as follows: special educational environment, problems of admission of the students as a member of the surgical team in operating room, and different culture of the operating room. By comparing these, we can say that the certain circumstances, difference of the operating room, a shortage of faculty member in the department, and less control of the trainer in the clinical environment of the operating room have affected the training opportunities of students in this field.

Operating room (OR) clinical practice involves special contextual elements related to the preoperative nursing process, practical OR nursing skills and teamwork in different patient care situations. However, the OR is one of most inaccessible places in a hospital where the use of space is highly regulated. Areas of space are divided into unrestricted, semi restricted and restricted sections, and the physical activities performed within each of these designated areas are differentiated (Riley and Manias 2002). Working conditions for graduates of surgical technology is different than those of other nurses. He is forced to comply with a variety of procedures and high workload quickly and carefully. Due to the quick entry and exit of patients, his work is not expectable and in many cases he is faced with acute and emergency situations and concurrent interactions with members of the surgical team. It can harden the clinical education of the students (Farneya et al 2013).

Because of the special atmosphere of the operating room compared to other sectors, it seems that there is more emphasis on the students of the field in technical skills and specific approaches of the care. In Khazayi's study (2015) about the clinical education of surgical technology, the students gave the highest points to the learning opportunities and the lowest points to the performance of educators and educational facilities (Khazaei et al 2014). The high point to learning opportunities is due to the favorable reception in parts that were faced with shortage of human resources and the students played the role of assistance in these sectors. In the study of Ghorbanian et al.(2014), the most important weaknesses of clinical education of operating room are as follows lack of using teaching aids, inadequate number of patients for learning, lack of knowledge of students of clinical evaluation system, lack of adequate oversight on the clinical education and lack of decision-making power of the student in planning the care of patients (Ghorbanian et al., 2014).

These are consistent with the contents obtained in our research. In the study of Dehqani et al (2007), the absence of proper scientific grounds, lack of interest in the students, confusion and uncertainty of the students in the absence of the trainer were mentioned as factors causing problem for training process (Dehghani et al., 2005). Omidvar et al in Babol University of Medical Sciences also considered the problems of clinical education related to health centers' personnel and their knowledge (Omidvar et al. 2005).

In a similar study by Hadizadeh et al., it was found that the understanding and cooperation of the health centers staff is important in the educational process (Hadizadeh et al., 2005). Providing diverse learning opportunities, receiving proper feedback from trainers as well as clinical environment are the important and effective factors of clinical training in the other country's studies (Andrews et al., 2006 and O'Driscoll et al., 2010). The results of this study showed that due to the special circumstances of the operating room, the admission of the students as a surgical team member has faced with difficulties.

Qualitative research of Lingard et al (2002) showed that the communication models in the operating room are complex and socially-motivated. The predominant themes include, time, security, sterilization, resources, roles and conditions. Often, every procedure had 1 to 4 stressful incidents and the stress transferred to other team members or the environment. In response to the tensions, the trainee of the operation was separated from the surgical team or began to mock. Both of these reactions are negative indicators for the team communications. The results are consistent with the study of Lingard et al (2002).

Tanner and Timmons (2000) emphasized that behavior in the OR is different from that in other hospital areas, and that the OR could be described as a 'backstage' area. In a backstage area interaction includes joking, talking about aspects of private life and behaving in a familiar manner. It also includes gossiping and disrespectful talking, even about patients during narcosis, which was also mentioned in this study. Frontstage behavior is the absence or opposite of this and occurs in the or when the patients are conscious before anesthesia or in the recovery unit (Tanner and Timmons 2000).Perhaps this antagonism between backstage and frontstage behaviors explains the students' excitement and frustration in the OR. Usually, before their OR placement period, students have spent time in frontstage areas, have dealt with staff more formally and have had closer caring relationships with patients. In addition, some nursing students have previous experience of patient care before enrolling in

the programme (Manninen 1998(which probably makes their views about nursing more frontstage-oriented

In this study, participants have considered the admission to the surgical team as a gateway to opportunities for learning clinical skills and using experience of team members. However, the admission has its own problems. In the study of Tazkori et al (2015), issues related to clinical education in the operating room are as follows, the lack of proper planning for training period, lack of trust in teachers, lack of flexibility in the face of criticism and non-compliance with professional ethics and lack of taking time for clinical and educational consultation, lack of learning opportunities in hospitals, lack of familiarity with the described tasks of the students. In Liponen's study (2004) which was conducted as descriptive phenomenology associated with the clinical setting of operation room, three types of teamwork were reported from the point of view of students:

Three types of teamwork emerged based on the students' perceptions: functional manifestation of OR teamwork, gaining OR team membership and technical orientation of OR teamwork. The findings are discussed in relation to OR practice, education and research on ways to improve teamwork while maintaining a satisfactory OR learning context and stimulating interest in perioperative nursing). (Silen-Lipponen, et al 2004) The implications of this study is consistent with the findings of our study. In another study that was conducted in Bushehr University of Medical Sciences, the results showed that the students consider the lack of learning opportunities as one of the educational barriers (Abidi and Motamed 2011).

Although working in the operating room is a teamwork and it needs participatory culture, the results of our study showed the hierarchical culture in the clinical setting of the operating room and the surgeon is the commander and decision maker and sometimes he/she loses the students' learning opportunities. Sadeghi et al (2011) believe that a weak and inflexible culture in the organizational environments causes employees not to tend to the innovation, change and creating new ideas and they fear from knowledge sharing. However, a dynamic, collaborative, and flexible culture reacts well to changes and leads to the progress (Sadeghi,,2011).

As studies show, participation and support from staff increase the learning opportunities for students (Heshmati et al2012). The results of another qualitative research (2004) showed that about 30 percent of the team transactions are faced with communication failure and cause problems. One-third of the cases associated with increased cognitive load (information processing in short-term and activated memory), interrupting the procedure, increased tensions and jeopardizing the safety of the patients (Zeighami et al 2004). However, Heshmatis study showed that nursing instructors use strategies like skills, realism, responsibility, determination, humility and using the experiences of nurses to attract the collaboration of clinical staff in providing clinical trainings (Heshmati 2012). However, other studies in our country showed that the nursing teachers have problems in playing their educational role (Heshmati et al 2010).

As stated by participants in this study, despite the difference and stressfulness of the clinical setting and its effect on clinical education of the students, participation and cooperation of every member of the surgical team can enhance the quality of the clinical skills of the students and this cannot be achieved unless in environments with supportive and participatory culture.This qualitative study was part of a larger study considering restriction of the studies with qualitative methodology; Such as: the subjective nature, field-based and their low level of generalizability. Despite these limitations, the results of this study provide deep understanding about the nature of clinical environment of the operating room and the problems related to clinical education of students of surgical technology.

CONCLUSION

According to the findings of this study, the unconventional educational environment in operating room, non-admission of the student in the surgical team as an educational member, and hierarchical culture of operating room have caused problems for these students. Since the work in the operating room is collaborative and concurrent, control of this clinical learning environment is difficult. In fact, clinical education of the surgical technology students is team-based and all members of the surgical team are, somehow, trainers in the shadow. Due to the dynamic nature of the operating room, cooperation of educational and clinical institutions can improve educational environment, admission of the student as an educational member in the surgical team, and creating a dynamic, collaborative and flexible culture.

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Effect of dynamic loading on removal torque value of one-piece and two-piece abutments

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ABSTRACT

The aim of this study was to assess the effect of dynamic loading and abutment type on removal torque value. Thirty-two analogs of fixtures with internal taper connections were divided into two groups of 16. The one-piece (OP) group received solid (one-piece) abutments and the two-piece (TP) group received two-piece abutments. Each group was further subdivided into subgroups C (control) without mechanical loading and T (test) with mechanical loading. The screw of abutments in OPC and TPC groups, were tighten and then removed to record the removal torque value (RTV). In OPT and TPT groups, abutments were tighten, mechanically loaded (300,000 cycles), removed, and the RTV were recorded. Two-way ANOVA and Tukey's HSD post-hoc testwere used for data analysis.The significance threshold was set at 0.05. The mean torque loss of OPC group was significantly lower than both TPC and OPT groups ($P < 0.05$). But there was not significant different in torque lossvalues between abutments in TPC and TPT groups.Under mechanical loading, theremoval torque of both one-piece and two-piece groups decreased and this reduction was only significant for one-piece group. Also, the abutment type has significant effect on removal torque value.

KEY WORDS: TORQUE, DENTAL ABUTMENT, CYCLIC LOADING

INTRODUCTION

Failure and complications with implant-supported prostheses still occur, despite the high clinical success rates in long-term. These complications include biological and mechanical problems. Screw loosening is the most commonly reported mechanical complication for single implant-supported prostheses. Different factors may

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contribute to loss or decrease in abutment screw torque such as fatigue, inappropriate tightening torque, failure in screw retightening after initial placement, settling effect, vibrating micro movements and excessive bending. The misfit of abutment or implant-supported crown, occlusal loading and structural design of implant connection are among other factors playing a role in this respect (Norton (1997, Jörnéus et al., 1992, Coelho et al., 2007 De Boever et al., 2006, Theoharidou et al., 2008).

The implant-abutment interface design can be roughly divided into two groups namely (I) butt-joints or slip fit joints with a passive fit and (II) conical interface with frictional fit between mating surfaces of abutment-implant complex; the latter type of interface also known as locking or Mores taper connection. In most previous studies, internal taper connections showed superior performance and were thought to minimize screw loosening and fracture, which commonly occur external hexagon butt-joint connections. An internal tapered abutment, based on the specific system used, may be attached to implant body by an external screw, orwith threads machined directly on the abutment body itself, which are calledone-piece (solid abutment) and two-piece abutments, respectively, (Finger et al., 2003 Budynas et al., 2008, Hansson 2000, Dittmer et al., 2011, Ricciardi et al., 2009, Cehreli et al., 2004 Aguirrebeitia et al., 2013 Rabelo et al., 2015).

It has been proven that both types are highly resistant to fatigue under dynamic loads and could function without any mechanical problems. One concern regarding the internal taper connection system is the possibility of cold welding of the abutment inside the implant. This was also mentioned by Sutter et al; whereas, other authors concluded that cold welding is presumably neutralized by a phenomenon called embedment relaxation. Clinically, it seems that One-piece abutments may be removed from the fixture more easily than two piece abutments, due to the nature of their design,which may causeless problems during removal, (Bozkaya et al., 1995, Weiss et al., 2000, Pintinha et al., 2013, Rabelo et al., 2015).

As mentioned earlier, screw loosening is the most common complication in single implant -supported restorations. This is important, particularly, incemented prosthesis, in which loosening or fracture of the abutment screw may lead to failure of prosthesis. Therefore, this study aimed to evaluate the effect of dynamic loading on removal torque value (RTV) of two types of internal taperabutments namely one-piece and two-piece abutments. The null hypothesis was that the RTV of one-piece and two-piece abutments would not decrease under dynamic loading.

MATERIALS AND METHODS

In this in vitro experimental study, 32 analogs of fixtures (Ø 4.8 mm* 10 mm L, Simple line II, Dentium, Korea) with internal taper hexagon were chosen. In addition, 32 abutments (11-degree taper, Ø 4.5 mm* 5.5 mm L, Simple line II, Dentium, Korea) of two different types (one-piece and two-pieces) were used. Each analog was mounted in a mold containing auto-polymerizing acrylic resin. The customized molds were fabricated from brass and measured 20 mm in height and 25 mm indiameter, in addition, their upper surfaces were cut so that this surface had 30˚ angle relative to the horizontal plane. Then, on the upper ramp, ahole was drilled perpendicular to the surface for placement of implant analog (Figure 1). This design of the mold allowed for the fatigue tester to apply load to the abutment at a 30 ˚ angle relative to the long axis. A wooden jig was used for correct positioning of the molds on the surveyor. The jig was a ramp with a 60 ˚ angle relative to the horizontal plane. Therefore, by assembling the mold on the jig its upper surface was positioned parallel to the horizon. Next, analogs were placed inside the hole perpendicular to the ramp of mold using a surveyor. The hole was filled with auto-polymerizing acrylic resin in doughy stage right before analog insertion (Figure 2). Then, analogs were divided into two groups according to the type of abutment they would receive. The study groups were as follows:

OPC group: One-piece abutments that were not subjected to dynamic loading (control group, n=8).
TPC group: Two-piece abutments that were not subjected to dynamic loading (control group, n=8).
OPT group: One-piece abutments that were subjected to dynamic loading (test group, n=8).
TPT group: Two-piece abutments that were subjected to dynamic loading (test group, n=8).

To measure the tightening and removal torques, a digital torque meter (TQ-8800; Lutron electronic, Taiwan) with an accuracy of precision of 0.1 Ncm was used.

FIGURE 1. Brass mold

FIGURE 2. Wooden jig for correct positioning of molds on the surveyor

Torque meter was positioned on top of a torque delivery device and the cylinder-analog-abutment assembly was fixed into a socket at the bottom of the device.

Before screw tightening, all abutments were lubricated with artificial saliva (Saliva Substitute; Roxane Laboratory Inc, USA). Then, all abutments in the control groups were tightened to 35 Ncm torque. After a 10-minute interval, abutments were retightened to the same torque to compensate for the loss of preload due to settling of surface at the interface. Ten minutes later, the RTV of abutments in the control groups was measured and recorded. For the test groups, 16 ceramic copings (e.max*Zir CAD, ivoclarvivodent) were fabricated with the same size and shape by computer aided design/computer aided manufacturing (CAD/CAM) technology (Sirona in Lab MCXL, Germany).

Since these abutments were cement-retained, for measurement of the RTV, the copings had to be removed from the abutment. For this reason, the coping were designed such that they had a hole in place of the abutment screw. Therefore, we hada direct accessto the coping hole, and there was no need to remove the crown after loading. Then, the adaptation of copings was verified and confirmed using light body silicone (Speedex, condensation polysiloxane, low consistency, Colten, Switzerland).

In the test groups, as well as the control groups, the abutments were torqued to 35 Ncmby digital torque meter in two cycles with 10-minutes intervals. Then, the crowns were placed on the abutments and cemented by a temporary cement (Temp bond, Kerr, Italy). During the experiment, the hole of crown was covered with composite. Afterward, each assembly of mold-analog-abutment-cap from the test groups was mounted and fixed to the electromechanical fatigue testing machine (CS-4, SDM echatronik, Germany) (Figure 3). The device has two lever arms that simultaneously apply force. The arm of device was so that the force was applied to the upper most part of the coping (Figure 4).

The fatigue tester was calibrated so that the lever arm soperated for 300,000 cycles (nearly corresponding toone year of chewing function) at a speed of 1Hz (60 rpm) [19].the position of load was Oblique load (withan angle of 30˚) of 100±5N was applied to each coping [19].

FIGURE 3. Dynamic fatigue tester

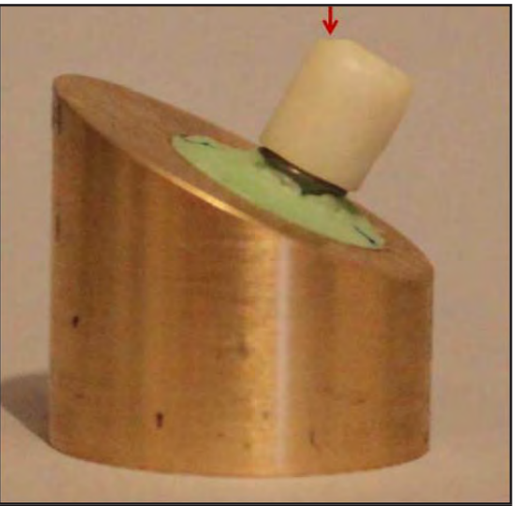

FIGURE 4. Ceramic coping and position of applying force

After each test, the specimens were transferred to the torque delivery device and the RTV was measured and recorded. The following formula was used to calculate the percentage of torque loss (Per tl):

$$Per\ tl = \frac{35 - removal\ torque}{35} \times 100$$

SPSS software version 21 was used for statistical analysis. The Per tl were statistically analyzed by Two-way ANOVA and Tukey's HSD post-hoc test. The significance threshold was set at 0.05.

RESULTS AND DISCUSSION

None of the tested samples showed abutment or screw fracture; there was no sign of crown loosening either after loading. Two-way ANOVA indicated that abutment types, dynamic loadingand their interaction had significant effects (P<0.05) on Percentage of torque loss. Table 1 shows the mean and standard deviations of Pertl inall study groups. There moval torque of abutments decreased in both groups (control and test group). TheOPC group presented the lowest torque loss. The highest torque loss was observed in OPT group. Pair wise comparisons of Pertl were then performed with Tukey's HSD post-hoc test (Table 2). The mean Pertl in abutments of OPC group was significantly lower than that in TPC and OPT groups (P<0.05). Also, the difference between OPT and TPT groups was significant (P<0.05), But there was no significant different in Per tl of abutments between TPC and TPT groups (P>0.05). In our study, all abutments in both control and test groups showed reduction in removal torque value compared to the insertion torque. Thisindicated that no cold welding occurred in any implant-abutment interface, which was consistent with the results of previous studies (Norton 1999, Ricciardi et al, 2009 Pintinha et al., 2013, Kim et al 2014).

Norton showed that cold welding occurs only at the highest level of torque, right before the component failure and when plastic deformation is expected. In addition, cold welding does not occurin clinical levels of torque, and the removal torque is expected to be 10 to 20 % less than the initial torque. However, Sutter and colleagues stated that following torque application, the removal torque increases from 10 to 15 % compared to the initial torque in internal taper connections. They argued that probably, the effect of axial component of the simulated occlusal force surpasses other oblique and tensile forces that interact negatively with retention of abutments. But, other authors have reported different results, indicating that the cold welding, if occurs, is compensated by the settling effect, (Ricciardi et al., 2009 Cehreli et al., 2004).

The results from One-piece abutments showed that the mean torque loss Inthe test group was significantly higher than that in control group (8.93% in the control and 51.02 % in the test group). In One-piece systems, abutment serves asa screw; therefore, in the test group, with application of dynamic load, these forcesare directly transferred to the threads and decrease the removal torque. In addition, the bending and tensile stresses are produced at the interface andlead to greater reduction

Table 1. Mean and standard deviation of per tl for all groups (n=8)

Group	N	Mean±standard deviation	95% confidence interval for the mean Lower bound	Upper bound	P value
OPC	8	8.93±4.76	4.9533	12.9167	.000
OPT	8	51.02 ±4.61	47.1711	54.8839	
TPC	8	22.8±8.00	16.1642	29.5408	
TPT	8	23.2± 8.97	15.8487	30.5538	

Table 2. Results of Tukey's HSD post-hoc test for pairwise comparisonsper tl

	Mean Difference (I-J)	95% Confidence Interval		
		Lower Bound	Upper Bound	P value
OPC& OPT	-42.092*	-51.3843	-32.8007	.000
OPC&TPC	-13.917*	-23.2093	-4.6257	.002
OPC&TPT	-14.266*	-23.5581	-4.9744	.001
OPT&TPC	28.175*	18.8832	37.4668	.000
OPT&TPT	27.826*	18.5344	37.1181	.000
TPC&TPT	-0.34875	-8.9431	9.6406	1.000

*The mean difference is significant at the 0.05 level.

oftorque. The results of a previous study showed various ranges of torque loss in One-piece abutments. Pinhata et al. reported the mean torque loss values of 18.35% for the control group and 15% for the test group, Pintinha et al. (2013). This value was between 15 and 20% in the study by Norton et al. and between 10.5 and 5.4% in the study by Ricciardi et al. (2013).

A mean torque reduction of 8% by Cehreli et al. (2004) and 25% by Seol et al. (2015) has been reported in test groups. Different results of studies can partly be related to differences in the types of implant systems used. In addition, differences in experimental conditions should be considered. There are significant differences between our study and others in number of cycles, intensity, position, angle and rate of applied force. In two-piece abutments, the difference between the control (with 22.8% torque loss) and test group (with 23.2% torque loss) was not statistically significant.

Pinhata et al. (2013) reported a mean torque loss of 36% and 40.85% for the control and test groups, respectively. Similarly, Ricciardi et al. 2013 found the mean values of 32% forthe control group and 37.2% for the test group.Also, Seol et al. (2015) reported a mean torque loss of 48% for test group.Based on the results of our study, in the control groups, one-piece abutments showed significantly higher removal torque value than two-pieceabutments. When opening one-piece abutments, the retention caused by the tapered part of the abutment as well as retention caused by the threads should be overcome. But intwo-piece abutments, the removal torque recorded by torque meter is mainly spent to overcome the retention friction generated by the threads because in two-piece abutments, the abutment screw passes through the abutment andat the time of opening, this unit is removed from the abutment. Therefore, in these types of abutments, a large amount of torque required by the tapered part of the abutment is not registered by the torque meter (particularly inabutments that have anti-rotation feature). Thus, it seems logical that two-piece abutments show less removal torque than one-piece abutments.

After applying force, the torque loss of one-piece abutments was significantly higher than that of two-piece abutments. In two-piece abutments, since the screw and abutment are in two distinct parts (yet related), smaller amount of force exerted on the abutment is transferred to thescrew; whereas, in one-piece abutments, as mentioned earlier, the abutment serves as a screw and transfers dynamic forces directly to the threads and decreases the required removal torque.

In this study, we tried to establish conditions to simulate clinical masticatory conditions. Each sample under went 300,000 cycles of dynamic force, which corresponded to one year of normal chewing function. Inaddition, the abutments were lubricated by artificial saliva before applying torque because it has been suggested that greater initial preload can be achieved underwet conditions, Jaarda et al., (1993). Siamos et al., 2002 Lee et al., 2002, Winkler et al., 2003)

Applying proper torque recommended by the manufacturer is very important to prevent screw loosening and screw fracture. Jaarda et al. reported 15 to 48% error when closing the abutment screw by hand. Therefore, the abutments were tightened to 35 Ncm torque by a digital torque meter. Ten minutes later, the same torque was applied to compensate the loss of preload due to settling effect. Siamos et al. suggested that in order tominimize the loss of preload caused by the settling effect, the initial torque should beapplied again 10 minutes after initial tightening torque, (Siamos et al., 2002).

Considering the fact that biteforce actually acts on the super structure, it was appropriate to perform anexperiment in which dynamic forces are applied on the abutment after cementation of crown. Before cementation, precise and passive fit of caps was assessed by light-body silicon. Prostheses with active fit or improper adaptation can exertun desirable forces on the abutment, (Lee et al. 2002).

The limitations of this study included small sample size and short-term loading. In addition, our study had an in vitro design and had the limitation of in vitro studies in simulating the complex nature of mastication cycles. In the oral environment, forces are applied in different directions and angles to the axis of abutments; moreover, the intensity of these forces is variable in different situations. The maximum biting force has beenreported in the range of 200 to 3500 N, (Winkler et al. 2003). But in the present study, a force of 100 N was applied, which is at the low end of this range. Also, the rate of force in this study was one hit per second, which was continuously applied within 3 to 4 days, but in normal oral function, 300,000 cycles of force are applied in a much longer period (aboutone year). All these factors can affect the behavior of screw and its loosening.

CONCLUSION

Under mechanical loading, RTV of both groups (one-piece and two-piece) decreased and this reduction was significant for one-piece group. However, there was no significant difference in RTV between one-piece and two-piece abutments under dynamic loading.

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Assessment of location of anterior ethmoidal artery using cone beam computed tomography (CBCT)

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ABSTRACT

Anterior ethmoidal artery (AEA) is an important landmark, which may be accidentally injured or traumatized during surgical procedures. This study aimed to determine the position of AEA on CBCTscans. In this descriptive analytical study, CBCT scans of 129 patients evaluated. The mean distance from AEA to anterior base of the skull, posterior frontal sinus wall, nasion, anterior nasal spine (ANS), orbital roof, inferior turbinate and the mean angle between AEA and ANS were evaluated using SIDEXIS 3D software. Independent and paired t-test and Fisher's test were used for statistical analyses with P<0.05 level of significance. The mean distance from AEA was 11.60±1.94 mm to orbital roof, 32.66±4.33 mm to inferior turbinate, 8.96±4.51 to posterior wall of the frontal sinus, 2.09 ±2.18mm to the anterior base of the skull, 18.01±3.90 mm to the nasion and 58.69±4.24 mm to the ANS. The angle between AEA and NS was 55.15±4.36°. The distances from AEA to orbital roof, inferior turbinate, nasion and ANS in men were significantly greater than those in women (P<0.05). nasion. (α = 0/05) Surgical procedures in areas close to AEA are high-risk. Determination of the position of AEA prior to endoscopic surgery of the sinus is highly important. CBCT can help determine the location of AEA prior to surgery to prevent unwanted surgical complications.

KEY WORDS: CONE-BEAM COMPUTED TOMOGRAPHY; ETHMOID; ARTERY

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INTRODUCTION

Inflammatory diseases of paranasal sinuses are a common serious problem. In the recent decades, functional endoscopic sinus surgery (FESS) has revolutionized surgical treatment of paranasal sinuses. In this technique, due to the proximity of paranasal sinuses to some important structures such as anterior base of skull, orbit and ethmoidal artery, unintentional injury to these anatomical structures may cause serious complications (Basak et al. 1998). Anterior ethmoidal artery (AEA) is an important landmark, which may be accidentally traumatized or injured during surgical procedure of anterior ethmoidal sinus. Injury to AEA may cause severe nasal bleeding, intra-orbital hemorrhage, posterior orbital hematoma, spinal fluid leak and intracranial bleeding in rare cases (Ding et al. 2012 Eren et al. 2016).

In FESS, superior lateral border of AEA and its inferior border in contact with inferior turbinate are marked (Rontal et al. 1991). The distance between AEA and the contact area with the inferior turbinate outlines the safe area for surgical manipulation. Since in FESS the width of ethmoid forceps is maximally 10mm when opened, the surgeon must have adequate knowledge about the space limitations in this area (Rontal et al. 1991; Basak et al. 1998). Knowledge about the anatomical location of AEA and its detection on images can help prevent unwanted complications during surgery (Ding et al. 2012). AEA is among the most important reference landmarks for endoscopic surgery since it determines the posterior location of recess. Its detection in frontoethmoidal suture determines the location of anterior base of skull (Monjas-Cánovas et al. 2012; Ko et al. 2014).

Ethmoidal artery originates from the orbital artery. Some branches of ethmoidal artery originate from supraclinoid portion of the internal carotid artery and pass through the optical foramen towards the orbit in a lateral position. In coronal plane, a conical groove in the medial orbital wall may be seen, which indicates the exit point of ethmoidal artery between the superior oblique muscle and medial rectus muscle. It indicates anterior ethmoidal foramen and can serve as a reference anatomical landmark to determine the location of AEA (Monjas-Cánovas et al. 2012). The location of AEA relative to the ethmoidal air cells may be variable but it is often located between the second and third lamellae next to basal lamella (Ding et al. 2012).

Ko et al, in 2014 evaluated the efficacy of AEA to determine the location of frontal sinus in endoscopic sinus surgery using computed tomography (CT) and offered a classification for the position of AE. Guldner et al. provided a similar classification in 2012 using CBCT (Guldner et al. 2012). At present, CBCT is increasingly used for implant imaging and endodontic treatments and is a new technique in ENT for assessment of sinuses. It is superior to CT since it has lower cost and lower patient radiation dose compared to CT. Studies on determination of location of AEA using CBCT are limited. Thus, this study has aimed to determine the position of AEA on CBCT scans.

MATERIALS AND METHODS

This descriptive analytical study was conducted on 129 CBCT scans of patients (70 females and 59 males) over 18 years of age presenting to the Oral and Maxillofacial Radiology Department of Isfahan University of Medical Sciences, School of Dentistry. All CBCT scans had been taken with one CBCT unit (Galileos-Sirona Bensheim, Germany) with the exposure settings of 85 kVp and 21-35 mAs (depending on the patient's weight). The inclusion criterion was age over 18 years. The exclusion criteria were history of surgery or trauma to paranasal sinuses and skull base, congenital facial anomalies, malignancies or lesions causing opacity of frontal recess and anterior ethmoidal cells. Multiplanar (coronal, sagittal and oblique sagittal) reconstructed images were assessed using SIDEXIS 3D software. Next, notch in the medial orbital wall and bony sulcus of lateral wall of the olfactory fossa (anterior ethmoidal sulcus) on coronal sections which indicate the anterior ethmoidal foramen were used for detection of the AEA (Figure 1).

Due to the presence of variations in the path of artery, distance from the artery to the base of skull was measured. In type I, the entry point of AEA is adjacent to the anterior base of the skull. In type II, it has less than 1mm distance from the base of the skull. In type III, AEA has a distance equal or more than 1mm from the anterior base of the skull (Guldner et al. 2012) (Figure 2). To determine the position of AEA relative to the frontal sinus in the sagittal plane, distance from the AEA to the posterior wall of the frontal sinus was measured (Monjas-Cánovas et al. 2011) (Figure 3). Distance from the AEA to the orbital roof and inferior turbinate (site of attachment

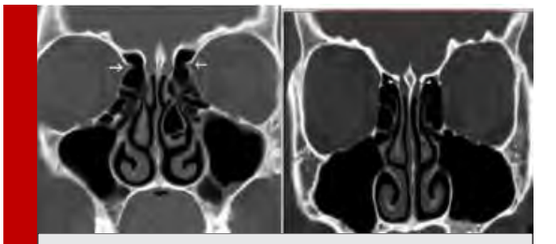

FIGURE 1. Anterior ethmoidal foramen notch in the medial orbital wall (arrow) and anterior ethmoidal sulcus (arrow head)

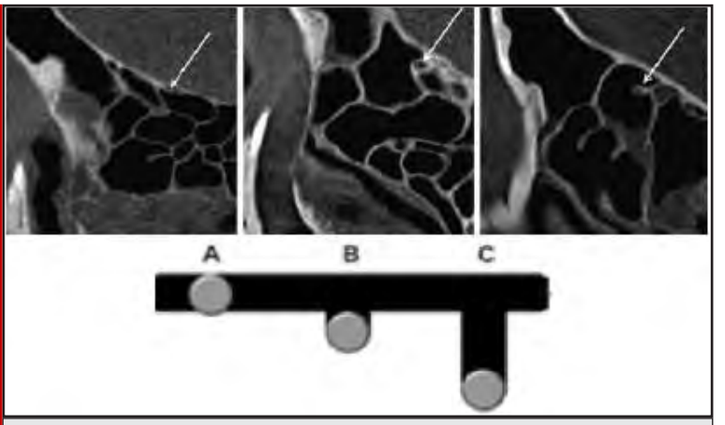

FIGURE 2. Location of the AEA on the sagittal plane (up). Schematic views (down): (A) Attached to the base of skull; (B) distance less than 1 mm; (C) distance of 1 mm or more

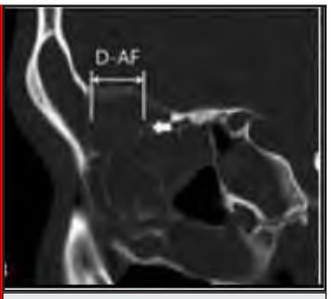

FIGURE 3. Distance from the anterior ethmoidal foramen to the posterior wall of the frontal sinus on the sagittal plane

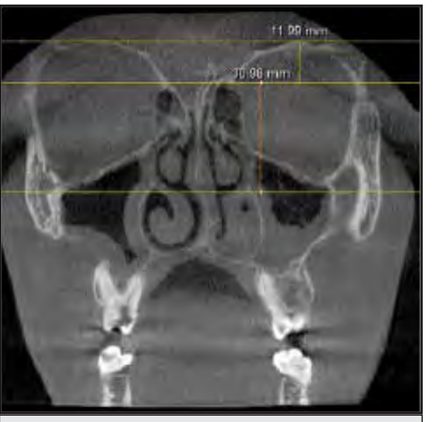

FIGURE 4. Distance from the anterior ethmoidal foramen to the orbital roof and inferior turbinate

of bony lamella of inferior concha with medial wall of the maxillary sinus) in the coronal plane (Figure 4) was measured. To estimate the distance from the AEA to the nasion and also the distance from the AEA to the ANS, the oblique sagittal plane was used. In this plane, the angle between the hypothetical line connecting the AEA and ANS and horizontal line passing through the hard palate and ANS was measured (Monjas-Canovas et al. 2011) (Figure 5). Independent and paired t-test were used for statistical analyses. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

A total of 129 patients (59 males and 70 females) with a mean age of 41.59 years in males and 38.44 years in females were evaluated. Both sides were evaluated in each patient (n=258). Independent t-test showed that the mean age of males and females was not significantly different (P=0.220). In most cases, the AEA was detectable on coronal sections of the anterior ethmoidal foramen in the medial wall of the orbit (96.1%) in the form of a notch and anterior ethmoidal sulcus in the lateral wall of the olfactory fossa (94.2%). The Fisher's exact test showed that the frequency distribution of presence of the AEA in the medial orbital wall and anterior ethmoidal sulcus was not significantly different in the right and left sides (P>0.05).The mean total distance from the AEA to the orbital roof on coronal sections was 11.60±1.94mm. This distance to the inferior turbinate was 32.66±4.33mm. As seen in Table 1, t-test showed no significant difference between the distance from the AEA to the orbital roof and to the inferior turbinate in the two sides (P>0.05). The mean distance from the AEA to the posterior frontal sinus wall (8.96±4.51mm) and anterior base of the skull (2.09±2.18mm) on sagittal sections is shown in Table 1; according to t-test, the differ-

FIGURE 5. Distance from the AEA to the nasion and ANS; angle between the AEA and ANS and hard palate plane on oblique sagittal plane

Table 1. Mean distance from the AEA to the orbital roof, inferior turbinate, posterior wall of the frontal sinus and anterior base of the skull in millimeters

Distances	Right (mean±SD) mm	Left (mean±SD) mm	Total (mean±SD) Mm	P value
Distance from AEA to orbital roof	11.73±2.03	11.48±1.85	11.60±1.94	0.13
Distance from AEA to inferior turbinate	32.42±4.46	32.90±4.21	32.66±4.33	0.20
Distance from AEA to posterior wall of frontal sinus	8.66±4.46	9.27±4.56	8.96±4.51	0.11
Distance from AEA to anterior base of skull	2.10±2.27	2.07±2.09	2.09±2.18	0.81

ence in the mean distance from the AEA to the posterior wall of the frontal sinus and anterior base of the skull was not significant (P>0.05).

Table 2 shows the position of the AEA relative to the anterior base of the skull on sagittal CBCT sections based on the presented classification.

Table 3 shows the mean distance from the AEA relative to the nasion and ANS and angle of AEA relative to ANS in oblique sagittal plane. As seen in Table 3, according to paired t-test, no significant difference was noted in the mean variables in the right and left sides (P>0.05).

As demonstrated in Table 4, independent t-test showed that the distance from the AEA to the orbital roof and inferior turbinate and distance from the AEA to the nasion and ANS in males were significantly greater than those in females (P<0.05) but no significant difference was noted between males and females in the AEA and ANS angle (P>0.05).

Many anatomical studies have assessed the location of the AEA and most of them used endoscopic measurements while some others used CT (Monjas-Canovas et al. 2012). On the other hand, studies on detection of AEA on CBCT scans are limited (Guldner et al. 2012).

Table 2. Location of AEA relative to the anterior base of the skull on CBCT sagittal sections in millimeters

	Right			Left			Total		
	0	<1	≥1	0	<1	≥1	0	<1	≥1
Number	58	1	70	50	2	77	108	3	147
Percentage	45%	8%	54.3%	38.8%	1.6%	59.7%	41.9%	1.2%	57%

Table 3. Mean distance from the AEA to the nasion, ANS and AEA-ANS angle on oblique sagittal plane

	Right (mean±SD)	Left (mean±SD)	Total (mean±SD)	P value
Distance between AEA and nasion	18.04±3.61 mm	17.95±4.17 mm	18.01±3.90 mm	0.78
Distance between AEA and ANS	58.58±4.09 mm	58.81±4.40 mm	58.69±4.24 mm	0.21
Angle between AEA and ANS	55.06±4.16 °	55.24±4.56 °	55.15±4.36°	0.53

Table 4. Mean and standard deviation of data based on gender

Distance	Female (mean±SD)	Male (mean±SD)	P value
Distance to orbital Roof	11.97±1.80 mm	11.17±2.01 mm	0.001
Distance to inferior turbinate	31.51±4.27 mm	34.03±4.02 mm	0.000
Distance to anterior base of skull	2.17±2.12 mm	1.99±2.25 mm	0.50
Distance to inferior wall of frontal sinus	8.56±3.83 mm	9.44±5.18 mm	0.132
Distance to nasion	17.25±3.28 mm	18.91±4.38 mm	0.01
Distance to ANS	56.46±3.33 mm	61.33±3.66 mm	0.00
Angle between AEA and ANS	55.44±4.06°	54.79±4.69°	0.23

The AEA passes through three cavities namely the orbit, the ethmoidal labyrinth and the anterior cranial fossa. The AEA enters into the olfactory fossa from the anterior cranial base and then enters into the anterior ethmoidal sulcus through the lateral lamella of the cribriform plate, which is the weakest point of the anterior base of the skull. At this point, bone is dangerously thin and it is considered a high-risk area in nasal endoscopic surgery. Thus, the AEA is susceptible to traumatization and injury during surgical procedures (Stammberger et al. 1990; Bayram et al. 2001; Moon et al. 2001; White et al. 2005; Araujo Filho et al. 2006; Souza et al. 2009).

In most cases in our study, this artery was detectable in the medial wall of the orbit (96.1%) and lateral wall of the olfactory fossa (94.2%). Similarly, Souza et al. detected AEA in the medial orbital wall (98%) and lateral wall of the olfactory fossa (100%) and stated that medial notch of the orbit and medial ethmoidal sulcus were reliable landmarks for detection of the AEA (Souza et al. 2009). Gotwald et al, similarly detected the orbital medial notch on coronal CT scans in 95% and medial ethmoidal sulcus in 84% of cases (Gotwald et al. 2004). Ding et al. used CT angiography and detected AEA in 100% of the cases in the notch in the medial orbital wall and in 98.4% in the ethmoidal sulcus (Ding et al. 2012). Considering the invasiveness of CT angiography and high dose of CT, low dose CBCT is more suitable for detection of AEA. In FESS, superior lateral border of the surgical site is determined based on the location of AEA and the inferior border is determined by the inferior turbinate (Rontal et al. 1991). Distance from the AEA to the attachment site of the inferior turbinate determines the suitable location for surgical procedure. Basak et al. considered the orbit as a fixed reference point and based on this point, they determined the location of AEA. The mean distance from the AEA to the orbital roof on CT scans was reported to be 13.7 mm (Basak et al. 1998) while in our study, this distance was found to be 11.60±1.94 mm. Basak et al. measured the mean distance from the AEA to the inferior turbinate to be 30.05 mm (Basak et al. 1998) and Eren et al. found this distance to be 31.6 mm on CT scans (Eren et al. 2016); these values were close to our findings (32.66±4.33 mm).

The distance from the AEA to the skull base is important because when AEA with mesenterium is located right beneath the skull, it has higher risk of traumatization during surgery. Basak et al. evaluated the location of AEA on coronal CT scans and reported that it was adjacent to the skull base in 57% of the cases and had a distance from it in 43%. In cases where AEA has a distance from the base of skull, risk of traumatization during FESS is higher (Basak et al. 1998). Moon et al. assessed both sagittal sections and cadaver dissection and reported that in 85.7% of cases, AEA was adjacent to the skull base; in the remaining cases, AEA had 2-3 mm distance from the skull base (Moon et al. 2001). Guldner et al. (2012) reported similar results. Araujo et al. reported that AEA was attached to the skull base in 83.3% of the cases (Araujo Filho et al. 2006). Kainz and Stammberger reported that in most cases, AEA had 5

mm distance from the skull base. They also stated that when the roof of ethmoidal sinus is low, anterior ethmoidal canal may be attached to the skull base (Kainz et al. 1988). The same was stated by Becker et al, who determined the location of foramen in endoscopic sections. Jang et al. reported this distance to be 1.32 ±1.51mm on CT scans (Jang et al. 2014) while in our study, this distance was 2.09 ±2.18mm. These differences may be due to racial differences or surgical technique. It seems that in patients with larger supraorbital ethmoid cell and optimal pneumatization of ethmoidal sinus (Jang et al. 2014), AEA is located right beneath the skull base; otherwise, it is at the same level of the skull base (Lisbona et al. 2010).

Young et al, in their study measured the distance from the AEA to the base of skull and categorized it in three groups of no distance [16%], distance less than 1 mm (33.7%) and equal or higher than 1 mm (50.5%) (Ko et al. 2014). In our study, 41.9% were categorized in no distance group, 1.2% were categorized in less than 1 mm group and 57% were categorized in 1 mm and higher group. In most cases in both studies, the distance from the AEA to the skull base was equal or more than 1 mm.

Frontal sinus is the most challenging sinus in endoscopic surgery due to its anatomical complexity and variations. Many studies have suggested anatomical landmarks to help the surgeon in safe conduction of frontal sinus surgery (McLaughlin et a;. 1997; Lee et al. 1997; Kew et al. 2002). The AEA is known as a typical classic reference point for the frontal sinus. Some studies showed that AEA is located right behind the frontal recess and can serve as an anatomical landmark for frontal sinus surgery (White et al. 2005). Thus, knowledge about the anatomy of this area is important and must be obtained prior to endoscopic sinus surgery. Young et al. reported the mean distance from the AEA to the posterior wall of the frontal sinus to be 8.58±5.56 mm, which was in line with our result (8.96±4.51 mm); whereas, Jang et al. measured the distance from the AEA to the anterior wall of the frontal sinus to be 17.36±2.19 mm [19]; this difference was due to the different reference points selected. Statistically, similar to our study, Young et al. found no significant association between the distance from the AEA to the frontal sinus and AEA to the base of skull (Ko et al. 2014).

In endoscopic surgery of the sinus, zero degree ANS angle complicates the detection of AEA [9]. Donemez et al. considered ANS as a fixed landmark and measured its distance from the AEA on cadavers using an electronic caliper, and reported the value to be 55±3 mm (Donmez et al. 2005). However, Monjas-Cánovas et al. measured the distance from the AEA to ANS to be 55.51±5.52 mm and measured its angle with the horizontal line passing through the ANS to be 57.7±1.78°; these values were close to those of Moon et al.(2001), Lee et al. (2000) and Araujo et al. (2006). Our study reported this distance to be 58.69±4.26 mm and the angle was 55.14±4.37°, which was in line with previous studies. Araujo et al. (2014) reported that the distance from the AEA to ANS was significantly greater in males than in females; this difference was not statistically significant in our study and that of Lee et al. (2000).

Nasion is used as a reference point in CT scan studies for surgical resection of some tumors or management of uncontrollable arterial hemorrhage, which is endoscopically impossible; although external incision is made from the canthus and not directly on the nasion. Monjas-Cánovas et al. measured the mean distance from the AEA to the nasion to be 29.31±2.5 mm (2011), which is similar to the study by Cankal et al. (2004); whereas, the results of Eren et al. (2014)(17.99±3.91) were closer to ours (18.01±3.90 mm). In our study, a weak but significant association was noted between the distance from the AEA to the ANS and the distance from the AEA to the nasion; whereas, Eren et al. (2014) found a significant association between the distance from the AEA to the nasion and the distance from the AEA to the inferior turbinate (Eren et al. 2014).

CONCLUSION

Inadequate knowledge about the anatomy of surgical site can cause serious iatrogenic surgical complications. To decrease these risks, a careful dissection must be done after thorough assessment of the area on CBCT scans. Therefore, adequate knowledge about the anatomy of the area and location of the AEA by use of CBCT can be helpful prior to FESS.

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Evaluation of two types of bleaching gel and light source on whitening of teeth

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ABSTRACT

Today, increasing desire for tooth brightening, make bleaching gel and light sources as a popular method for tooth whitening. The aim of this study was effect of combination of bleaching gel and light source on the teeth whitening. In this study 60 healthy human anterior teeth were randomly dividedin three groups (n=20). The first groupwas without-light, the second one LED andon the third group QTH light was used. Each group was divided into two subgroups whichhad two types of hydrogen peroxide bleaching gelsincluding 37.5% Pola office and 40% Opalescence Boost which were applied to them. The primary colors of teeth were measured, and then kept intea (200ml) for 7 days. After that each tooth color was measured for the second time. The teeth were bleached according to the protocol of each group: Immediately, after 3, 7 and 30 days they were measured by spectrophotometer. Data were analyzed by statistical software SPSS 20 and One-way ANOVA and Tukey testin alpha 0.05. Bleaching effectiveness of QTH significantly was increasedin both of bleaching gels as compared to LED and without using of light, at different times: instantly, 3 days and 7 days after bleaching, a month after bleaching, significant differences between the different methods was not seen. The results of this study showed thatin-office bleaching, with or without light is an effective method of beaching. QTH Light in the short term (one week) showed significant impact on teethwhitening in both bleach gel. However, within a month therewere no significant differences in all groups.

KEY WORDS: IN-OFFICE BLEACHING, HYDROGEN PEROXIDE, ACTIVATION LIGHT

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INTRODUCTION

In recent years, cosmetic dentistry has become an important part of restorative dentistry. In all ages,the teethappearances for patients are one of important aspects which influences on satisfaction of them.Desire of having a whiter tooth has increased due to attention of people to this novel that brighter teeth are healthier and morestylish (Barghi, 1998). The bleaching is very effective on teeth with high Hue and yellow tint (Ishikawa-Nagai, Terui, Ishibashi, Weber, & Ferguson, 2004). In fact, dental bleaching materials are oxidizing materials which difference in various techniques still stay controversial (Izquierdo-Barba, Torres-Rodríguez, Matesanz, & Vallet-Regí, 2015).

Today, several bleaching methods are used including: in-office professional, in-home professional (dentist supervised take-home) and products without a prescription (over-the-counter) (Caneppele, 2013). To speed up the bleaching process, increasing the concentration of chemicals or use different light devices recommended, (Sulieman, Addy, MacDonald, & Rees, 2004), (Buchalla & Attin, 2007). Some advantages of in-office techniques like prevention of swallowing and contacting with oral soft tissue, reducing treatment time and controlling of the healing process make it as a suitable technique for most patients compared to other techniques (Luk, Tam, & Hubert, 2004), (Tay, Kose, Loguercio, & Reis, 2009).

Two important factors in estimating the teeth whitening product performance are peroxide concentration and the duration of its application. Studies have shown that higher concentrations of peroxide is required a smaller amount of gel (Sulieman et al., 2004). In today's in-office bleaching carbamide peroxide and hydrogen peroxide are used mainly by heat or light-activated devices to accelerate the process of bleaching teeth (Sulieman et al., 2004), (Zhang et al., 2007). Most studies have suggested that light cure accelerated the degradation of peroxide (by increasing temperature) which led to theformations of higher free radicals(Sulieman et al., 2004), (Joiner, 2004), (Lima et al., 2009). today various light cure devices like Light-emitting diode(LED), halogen lamp, plasma arc lamp(PAC) are available, however, application of heat, light or laser devices should not be more than 5.5°C temperature due to preventing pulp chamber damage(Buchalla & Attin, 2007). Although, in recent years the use of laser bleaching is generally available as an energy source but LED require nearly low cost, and less energy (Kurachi, Tuboy, Magalhães, & Bagnato, 2001). There is controversy about the effectiveness of different light on teeth bleaching. although some researchers have reported benefits,however others showed no effects on bleaching, (Ishikawa-Nagai et al., 2004, Lima et al., 2009, Polydorou, Wirsching, Wokewitz, & Hahn, 2013).

Polydorou et al. (2013) have reported that QTH is more effective than laser bleaching, while Hahn et al. (2013) did not investigate any improvement with LED and laser in bleaching teeth. In another study which evaluated six different light resulted that the diode laser, QTH and LED had a significant impact on Teeth Whitening (Domínguez et al., 2011) Similarly, Kossatz et al., compared effect of LED and laser on 35% hydrogen peroxide bleaching gel and reported no significant differences (Kossatz et al., 2011).

In another study where 35% hydrogen peroxide was used, LED, QTH, plasma arc lamp, argon laser was used and they have been reported no effect on tooth bleaching (Lima et al., 2009). Clinician should be aware of any risks regarding to bleaching process (Alqahtani, 2014). The aim of this study was to evaluate effect of LED and QTH light on teeth whitening, since using of any type of light as an additional device is questionable in-office bleaching.

MATERIAL AND METHODS

In vitro experimental study, the 60 human anterior teeth were selected. First teeth were checked for any defects, cracks, decays or fillings. Then selected teeth after scaling, brushing (for 10 seconds with pumice and water by prophylaxis rubber cup then cleaned with ultrasonic cleaner, and stored for one week in the solution0.1% Thymol at 4°C. While the entire of laboratory work used gloves, surgical masks and face shields (Kohn et al., 2003), (Kumar, Sequeira, Peter, & Bhat, 2005), (Lolayekar, Bhat, & Bhat, 2007). The teeth were fixed in acrylic resin into the mold which the angle of light cure is perpendicular to the labial surface of the teeth. By creating four small holes (depth of about 0.5 mm) with bur (No#1) created square-shaped area (with dimensions of approximately 3 mm) in the middle third of the labial teeth (Figure 1).

Labial surface of teeth were cleansed and brushed. Then all the teeth stored in Ringer (Ringer's Infusion, Shahid Ghazi Pharmaceutical Co., Tabriz-Iran). The teeth are completely dry with gauze and randomly divided into three groups (20 = n) with different bleaching protocols:The first group without light, the second LED light cure system (Woodpecker Dental LED.D Curing Light, China), the third group QTH light cure system (Coltolux 75 Curing Light-Coltene/Whaledent, USA). Each group divided into two subgroups of 10 teeth the first subgroup, hydrogen peroxide 37.5% Polaoffice + (SDI, Australia) and in the second subgroup of hydrogen peroxide 40% Opalescence Boost (Ultradent Products Inc, South ordan, UT USA) was used (figure 1).

The samples were placed on holder plate, white paper Leneta. Light source positioned at an angle of 45 degrees

FIGURE 1. Preparation of tooth for bleaching

to a line perpendicular to the surface and spectrophotometer device (KONIKA MINOLTA CS2000, Japan) with an approximate angle of zero degrees relative to a line perpendicular to the sample surface and they were placed approximately 70 cm.

The device was set at 0.2 degrees. This angle makes circular area with 2.4 mm in diameter in specimens. Three measurements were taken for each sample and average measurements were reported. Measurements were conducted under laboratory conditions at approximately 25°C. The primary color of the teeth (M1) was measured by spectroradiometer. for changing the color, teeth were retained for 7 days in a mixture of tea (tea bags Golestan) obtained by the method Sulieman et al (Sulieman, Addy, & Rees, 2003). Teethwere brushed to remove pigments.

Then the teeth color was measured for the second time (M2). The teeth were bleached in all 3 groups according to the protocol set for each group, while the distance to the labial surface of the teeth were 5 mm to light tip. The intensity of light rays was measured before the start of each bleaching cycle using Radiometer (QTH = 370mw/cm2, LED = 380mw/cm2) to ensure constant light output is achieved at all stages of bleaching. In all three groups, the use of bleaching gel and light were performed exactly according to the manufacturer's instructions. A thin layer of (1 mm) Polaoffice bleaching gel was placed on the labial surface of the teeth for 8 minutes, then using clean cotton it was cleansed by gel then was stained with fresh gel.

This process was repeated three times (according to the manufacturer's instructions) and at the end all clear gel, rinsed with water and dried and the bleaching gel Opalescence Boost, a thin layer (mm1) of gel on the labial surface of teeth put for 20 minutes (according to the manufacturer's instructions) and at the end all clear gel, rinsed with water and dried. Light application in groups 2 and 3 were three 8-minute for Polaoffice and a 20-minute period to Opalescence Boost.

Tooth color was measured immediately after bleaching (M3). Samples were put on Ringer with 37 ° C which switching with new Ringer every day then he teeth color were measured after 72 hours (M4), 1 week (M5), one month (M6). All stages of tooth color measurement system were performed by spectrophotometer according to CIE L*a*b* (Commission Internationale de l'Eclairage's) (Tavares et al., 2003). According to the ADA (American Dental Association) tooth color measurement, device located in the middle third of the labial surface of the teeth (figure 2).

Tooth bleaching ΔE values are directly influenced by the index tooth color difference between the initial measurement and other measurements are shown and using the formula: E = [(ΔL *) 2+ (Δa *) 2+ (Δb *) 2] 1 / 2Δ (Δa = a * post - a * baseline, Δb = b * post - b * baseline, ΔL = L * post - L * baseline) was achieved. Data were collected with 20 SPSS statistical software and ANOVA test value of P (P<0.05) were obtained and studied under Tukey test was used pairs of groups.

RESULTS AND DISCUSSION

ΔE averages and standard deviations for all groups and in all stages after bleaching compared to after being in tea shown in Table 1.

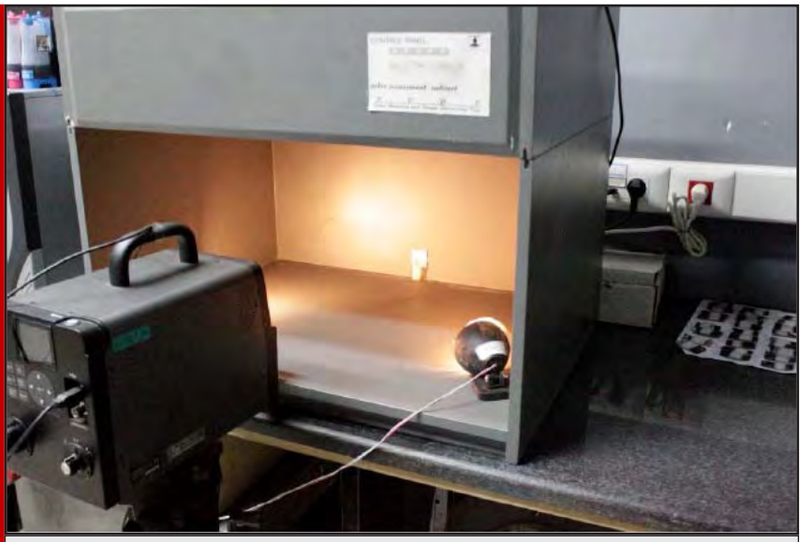

FIGURE 2. Evaluation of samples by spectrophotometer

Table 1.

1 month after bleaching	7days after bleaching	3 days after bleaching	Immediately after bleaching	Groups
6/74 (1/12)	3/92 (0/82)	4/95 (0/9)	3/8 (0/57)	Polaoffice+without light
9/83 (2/78)	3 (0/8)	3/82 (0/77)	2/89 (1)	Opalescence Boost without light
7/25 (0/88)	4/78 (0/49)	5/39 (0/58)	3/57 (0/56)	LED Polaoffice+
7/17 (1/08)	98/3 (0/53)	4/21 (0/37)	3/28 (0/54)	LED Opalescence Boost
6/58 (1)	10/84 (1/11)	10/3 (1/09)	8/82 (1/21)	QTH Polaoffice+
6/32 (0/81)	8/44 (0/45)	8/08 (0/45)	7/28 (0/4)	QTH+Opalecensce Boost

Significant difference between ΔE of all groups after being in tea using one-way ANOVA test was observed. (P = 0.47)

Significant difference between ΔE of groups (at times immediately, three days and one week after white) one-way ANOVA test was observed (P-Value <= 0.05). ΔE significant difference between the groups was observed one after month the bleaching. (P = 0.57)

Comparing of pairs of groups by test Tukey, both of bleaching, a significant difference in the effectiveness of bleaching (ΔE) with the use of light QTH compared to LED and without the use of light (in the time immediately after bleaching, three days and 7 days after bleaching) showed that although a month later bleaching of teeth whitening showedsignificant difference in efficacy between any of these groups (Table 2).

To achieve lightening of teeth in a short time, bleaching technique in the office, using a high concentration hydrogen peroxide gel, with and without the use of light is recommended (Luk et al., 2004), (Roberto et al., 2011). Some studies have emphasized that light can be used to catalyze the hydrogen peroxide, thus accelerates the bleaching process (Dostalova et al., 2004), (Tavares et al., 2003). This issue is still discussed in articles and reviews. In the present study, the teeth ΔE after tea, significantly increased (ΔE in all groups was higher than 3.3 means the discoloration caused by tea in all groups were detectable by eye) ΔE, difference before and after exposure to tea among all groups was not significant.

In this study, ΔE changes in groups (immediately, three days and one week after bleaching), was significant,so the applicationof Polaoffice with QTH showed whitening effect. In the present study, the Tukey test results showed that in both bleaching agent (Opalescence and Pola office), QTH Light application significantly increased the effectiveness of bleaching component compared to LED or without light, immediately after bleaching teeth, 3 days and 7 days after bleaching, Because QTH heat transfer capability to the teeth is more than LED. QTH produce more heat than light on the tooth surface which can form groups that cause more tooth dehydration (Liang et al., 2012). Also, previous studies which have examined different types of light reported that QTH effect was remarkably higher in immediately after bleaching (Sulieman et al., 2005, Polydorou et al., 2013 Liang et al., 2012).

Table 2.

P-value After one month	P-value After 7days	P-value After3 days	P-value Immediately	Groups
0.31	0. 44	0. 35	0.45	Polaoffice with Opalescence Boost Without light
0. 95	0. 28	0.1	0.71	Polaoffice with Opalescence Boost LED
0. 84	0. 08	0.08	0.24	Polaoffice with Opalescence Boost QTH
0. 93	0. 75	0.93	0.98	Without light Polaoffice + LED Polaoffice+
0. 99	*0.00	*0.001	*0.001	Without light Polaoffice + QTH + Polaoffice
0. 89	*0.00	*0.002	*0.00	Polaoffice + LED with QTH + Polaoffice +
0. 55	0.5	0.87	0.92	Without light + Opalescence Boost LED + Opalescence Boost
0. 36	*0.00	*0.00	*0.001	Whithout light + Opalescence Boost QTH + Opalescence Boost
0. 94	*0.00	*0.00	*0.001	LED + Opalescence Boost QTH + Opalescence Boost

ΔE Changes in groups at different times after bleach in arein Charts 1, 2 and 3.

In this study, ΔE difference was not statistically significant a month after bleaching,in all groups, ΔE was more than 3.3 which teeth bleaching was still detectable after a month with eye. Tukey test results compare pairs of groups after one month however no significant difference was observed between ΔE. This means that after a month no statistically significant differences between the different methods of bleaching as observed similar results from studies have concluded the same, (Polydorou et al., 2013) and (Alomari & El Daraa, 2010).

In this study, all groups showed obvious effect immediately after bleaching, though there is noconsiderably differences between one and three months. Although some studies have reported the effects of light on tooth whitening (Luk et al., 2004), (Domínguez et al., 2011), (Ontiveros & Paravina, 2009), (Wetter, Barroso, & Pelino, 2004), but many studies have focused Light extremely ineffectiveness on teeth whitening (Lima et al., 2009), (Polydorou et al., 2013), (Roberto et al., 2011),(Bernardon et al., 2010), (Marson, Sensi, Vieira, & Araújo, 2008), (Mondelli, Francisconi, Almeida, & Ishikiriama, 2012), (Hein et al., 2003), (Papathanasiou, Kastali, Perry, & Kugel, 2002).

Although, in this study QTH had greater effectiveness after 7 days, but after one month treatment relapse was observed. In Polydorous study, the use of QTH for each tooth was 4times in 8 minutes (Polydorou et al., 2013), while in Liangs study, two sessions with7 days interval 3 times of 10 minute were applied (Liang et al., 2012). So in both of these study groups, teeth were exposed longer to QTH light. Relapse in treatmentin the Polydorous study only occurred in a month after treatment QTH group (Polydorou et al., 2013), which is similar to the results of our study, while both groups in the Liangs study showed relapse after a week with or without light cure system (Liang et al., 2012).

For other groupsin the Polydorous study, relapse occurred after 3 months (Polydorou et al., 2013), while in Liangs it happened sooner (Liang et al., 2012). These results can be explained by a lower concentration of bleaching agent used in study (32% Beyond II Advanced Formula Whitening Gel) compared with current study (37.5% Polaoffice and 40% Opalescence Boost) and Polydorou study (38% Opalescence Boost), although this difference is negligible. Tooth dehydration can cause brighter teeth immediately after bleaching so this can be increased by the heat transferred to the teeth (Luk et al., 2004). Tooth color becomes lighter during dehydration however returning to normal mode after rehydration. During in-office bleaching, many factors could cause dehydration teeth, including teeth isolation, heat from the light (Liang et al., 2012).

According to the results of this study at any time after the teeth bleaching, a significant difference in the effectiveness of the bleaching was observed regardless of light cure application, Which could be due to the concentration of these two bleaching gel (37.5% Pola office + and 40% Opalescence Boost). Also, according to the manufacturer's instructions, each of bleaching gel which hadless concentrations, longer exposure in the tooth surface (37.5% Polaoffice 3 times for 8 minutes and 38% Opalescence Boost only 20 minutes)so the effectiveness of bleaching materials weresimilar. Scientific community believe that light cure bleaching effectiveness occurs due to reversible dehydration (Polydorou et al., 2013) so in long-term we should expect relapse of treatment. The results obtained in this study during a month is not only confirmed the stability of tooth

color But also enhances the white color of the teeth in both LED and without light cure system. The Greenwall hypothesis is that during bleaching, teeth get aerated by oxygen and oxidation processes caused by dehydration change the visual characteristics of the teeth. This partly is explained why teeth become whiter in this study after a month. For this reason, after a two-week period, scattered oxygen rehydrate and teeth show their true colors (Greenwall, 2001).

While in current study, the majority of teeth bleached within a month except QTH group which heat lead to improve the effectiveness of treatment in few first days,another parameter that can affect the outcome of the study is the viscosity of bleaching gel. Opalescence Boost was replaced with Opalescence Xtra Boost some time ago, although, manufacturer claim no chemical change, but the foundation and consistency has changed; the new bleaching gel is more viscous than before. The composition and sticky bleaching gel affect on the release of hydrogen peroxide which impact on bleaching process (Christian Hannig, Weinhold, Becker, & Attin, 2011), (Hannig, Zech, Henze, Dreier, & Attin, 2005), (Thitinanthapan, Satamanont, & Vongsavan, 1999). high viscosity bleaching agents compared to low one shows higher peroxide emissions (Christian Hannig et al., 2011).

This change in viscosity after bleaching can cause different behavior because the viscosity of the material affects the free movement of peroxide (peroxide release kinetics). The clinical perspective, highlights light cure effect on the confidence and satisfaction of patients to continue bleaching. After a month there was no significant difference between the three groups and this shows that bleaching by light relapse sooner and do not maintain long-term effect.

CONCLUSION

According to data obtained from this study, the following results can be obtained. In office- bleaching, with and without light, is effective to bleach teeth. In both type of bleaching, bleaching effectiveness of QTH increases significantly compared to LED and without the use of light, at times of instantly, 3 days and 7 days after bleaching.Light cure does not lead to increase effectiveness of bleachingwithin a month.

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Area-metric analysis of the quality of obturation of four different techniques: An ex vivo study

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ABSTRACT

The aim of this study was to compare different obturation techniques in straight canals in term of the percentage of gutta-percha filled area (PGFA) and percentage of sealer filled area (PSFA) and voids. The obturation time was also compared. Straight root canals of 40 extracted human premolars were instrumented with Mtwo rotary files and filled as follows: Control: cold lateral compaction with standard master and accessory cones. CLS group: cold lateral compaction with 0.04/35 master cone and standard accessory cones. CLNS group: cold lateral compaction with 0.04/35 master cone and non-standard medium-fine (MF) accessory cones. WLS group: warm lateral compaction with 0.04/35 master cone and standard accessory cones. AH26 sealer was used. The teeth were sectioned horizontally at 3, 6 and 9 mm from the apex. The total area of each section and the PGFA, PSFA and percentage of voids were calculated. Data were analyzed using repeated measures ANOVA and Kruskal-Wallis test.The CLNS was the fastest method with a significant difference with the control group ($P<0.05$) while WLS required significantly more time than all other techniques ($P<0.05$). In the apical and coronal thirds, CLNS yielded significantly lower PGFA ($P<0.05$). In the middle third, WLS yielded significantly higher PGFA than CLNS ($P<0.05$). Cold lateral compaction using gutta-percha master cone with 4% taper and MF accessory cones is not efficient for obturation of canals. Also, warm lateral compaction has no superiority to cold lateral compaction in straight canals.

KEY WORDS: COLD LATERAL CONDENSATION, SEALER, WARM LATERAL COMPACTION, OBTURATION, GUTTA-PERCHA

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INTRODUCTION

Achieving a hermetic apical, lateral and coronal seal is an important factor to prevent entry of microorganisms into the canal and periapical tissues, and depends on proper cleaning and shaping and efficient obturation of root canal system (Wu et al. 1993; Mollander et al. 1998; Schafer et al. 2012). Advances in formulations of root canal irrigating solutions and rotary devices have greatly enhanced proper cleaning and shaping of root canal system. Homogenous, three-dimensional filling of root canal space by gutta-percha and sealer can greatly increase the success of endodontic treatment (Wu et al. 2002). Gutta-percha cones must be compacted next to each other with a very thin layer of sealer between gutta-percha cones and canal wall to minimize voids (Gordon et al. 2005). Gutta-percha dimensions do not change over time (Wu et al. 2009) but sealer undergoes shrinkage and dissolution (Al-Hadlaq et al. 2010; Nica et al. 2012). Following dissolution of sealer over time, voids are created in the canal, which can compromise the seal and enable entry and accumulation of microorganisms and subsequent development of periapical lesions and eventual treatment failure (Jarret et al. 2004; Schafer et al. 2012).

If the volume of gutta-percha mass compared to that of sealer increases, less voids will be created over time and coronal and apical seal will not be compromised. This would increase success of endodontic treatment (Kazemi et al. 1993; Kontakiotis et al. 1997; Wu et al. 2009). A systematic review reported that canals filled with adequate, homogenous gutta-percha mass showed primary endodontic success (Ng et al. 2008). Another study reported that homogeneity of gutta-percha mass had a significant positive effect on periapical health and success of endodontic treatment (Lee et al. 2012).

Root canal filling length should be equal to the working length to ensure that the entire prepared space is filled with gutta-percha (Kojima et al. 2004; Schaeffer et al. 2005). Thus, maximum penetration of master and accessory gutta-percha cones must be ensured. The taper of master and accessory gutta-percha cones is an important factor affecting the penetration depth of gutta-percha into the canal such that the greater the compatibility of taper of gutta-percha with that of root canal, the more homogenous and regular their arrangement would be in the canal. Some authors believe that using a single gutta-percha cone that matches the taper and size of prepared canal would have the best adaptation to canal walls (Whitworth, 2005; Schafer et al. 2013).

Several methods have been suggested for root canal obturation such as lateral compaction, vertical condensation, single-cone obturation with a gutta-percha cone that matches the size and taper of prepared canal (Schafer et al. 2012), use of a solid core covered with gutta-percha, injection of soft, plasticized gutta-percha into the canal and use of physically and chemically modified gutta-percha with higher adaptation (Skinner et al. 1987; Whitworth, 2005; Schafer et al. 2013). Each of these techniques has its own advantages and disadvantages, and no consensus has been reached on the superiority of one technique over the others (Aqrabawi et al. 2006; Ng et al. 2007).

Lateral compaction is a commonly used root canal obturation method due to its simplicity (Cailleteau et al. 2009). This technique can be practiced in most clinical situations and enables length control during compaction (Gilhooly et al. 2001). However, its main drawback is that it cannot create a homogenous mono-block of gutta-percha. Master and accessory gutta-percha cones are placed next to each other but remain separate and the space between cones is expected to be filled with sealer, which undergoes shrinkage and dissolution over time. In thermoplastic methods such as warm vertical condensation and warm lateral compaction, heat is used to cause physical changes in gutta-percha and increase its plasticity and adaptation to the canal walls. Warm lateral compaction allows length control and at the same time, heat results in adherence of gutta-percha cones to each other and formation of a solid mass (Jacobsen et al. 1993).

Considering the need for decreasing working time in dental office, gutta-percha cones with 4% and 6% tapers and non-standard medium fine (MF) and fine-fine gutta-percha cones were introduced to the market for faster obturation of root canals. However, deep penetration of accessory cones with greater taper is difficult. Studies on penetration depth of spreader alongside a master cone with 2% taper and master cones with higher than 2% taper showed greater penetration depth of spreader next to master cone with 2% taper (Bal et al. 2001; Wilson et al. 2003). Thus, complete penetration of accessory cones to the working length and obtaining an apical seal with the use of these gutta-percha cones is a matter of debate. This study has aimed to assess the quality of root canal filling by measuring the percentage of gutta-percha (PGFA) and sealer filled areas (PSFA) and voids in use of four different obturation techniques. Duration of obturation was also compared among the four methods.

MATERIAL AND METHODS

This in vitro, experimental study was conducted on 40 single-canal maxillary and mandibular premolars with straight canals. The teeth were randomly divided into four groups (n=10). The highest mean difference expected for PGFA among the groups was considered to

be 0.13 with a standard deviation of 14% according to a study by Schafer et al. in 2012. Considering 80% power of study and type one error of 5%, sample size was calculated to be 10 in each group.

The inclusion criteria were single-rooted premolar teeth extracted for orthodontic or periodontal reasons with a straight canal and one apical foramen and no visible root caries, no fracture or crack and no internal/external root resorption or calcification on radiographs. The teeth had mature apices.

The exclusion criteria were presence of voids after filling and incorrect working length. Teeth with no apical constriction or fracture during cleaning and shaping were also excluded. After collection, the teeth were immersed in 3% sodium hypochlorite solution (Rakhshan, Tehran, Iran) at room temperature for two days. Calculus (if any) was removed using a universal curette (Hu-Friedy, USA) and the teeth were evaluated under a stereomicroscope (SZX9; Olympus, Tokyo, Japan) at 20 magnification to ensure absence of root cracks. Parallel periapical radiographs were obtained in mesiodistal and buccolingual dimensions to assess root canal anatomy. After access cavity preparation using 836 fissure and 801 round burs (SWS, Switzerland), a #15 K file (Mani, Tokyo, Japan) was introduced into the canal until its tip was visible at the apex. The teeth were then radiographed and canal curvature was determined. The selected 40 teeth had straight canals and #20 K file was the largest initial file compatible with their root canal. The crowns were then cut at the cementoenamel junction using a diamond disc (D & Z, Germany).

To determine the working length, a #15 K file (Mani, Japan) was introduced into the canal such that its tip was visible at the apex; working length was determined 1mm short of this length. All canals were cleaned using #15 and #20 K files (Mani, Japan) and then by Mtwo rotary NiTi files (VDW, Germany) with an endodontic motor (NSK, Japan) with slow pull and push brushing motion to the working length as recommended by the manufacturer in the following order: 0.04/10, 0.05/15, 0.06/20, 0.06/25, 0.05/30, 0.04/35. A #10 K file (Mani, Japan) was used after each rotary file for patency and 17% EDTA gel (Calasept, Nordiska Dental) was used as chelator. Root canals were rinsed with 2cc of 1.5% sodium hypochlorite (Rkhshan, Tehran, Iran). Smear layer was removed by rinsing the canals with 5cc of 17% EDTA for one minute followed by a final rinse with saline. The root canals were dried with paper points (Ariadent, Tehran, Iran) and the teeth were randomly divided into four groups of 10 and filled with AH26 sealer (Dentsply DeTrey-Germany) and gutta-percha (Metabiomed, Korea) as follows:Control group: #35 standard gutta-percha (2% taper) as master cone and standard accessory cones were used in cold lateral compaction technique.

CLS groups: #35 standard gutta-percha (4% taper) as master cone and standard accessory cones were used in cold lateral compaction technique (cold lateral compaction with standard gutta-percha).CLNS group: #35 standard gutta-percha (4% taper) as master cone and non-standard MF accessory cones were used in cold lateral compaction technique (cold lateral compaction with non-standard gutta-percha).WLS group: #35 standard gutta-percha (2% taper) as master cone and standard accessory cones were used in warm lateral compaction technique (warm lateral compaction with standard gutta-percha).Root canal filling with cold lateral compaction technique was done as follows: Following root canal preparation and drying, a standard gutta-percha cone with 2% taper was used in the control group and with 4% taper in the CLS group to the working length. Apical third of master cone was dipped in sealer and placed into the canal, rotated in counter clockwise fashion and removed. A proper size spreader was also selected based on canal taper to reach 1mm of the working length with master cone in the canal. Accessory cones were placed and cut at the orifice. No pressure was applied for vertical condensation.

Cold lateral compaction in CLNS group was the same as in the control and CLS groups with the exception of 4% taper of master cone and selected spreader. Also, non-standard MF accessory cones were used. In WLS group, the same procedures were followed with the exception that after placement of master cone with 4% taper, the thinnest tip of Obtura (E & Q Master Meta Biomed-Korea) that could reach 2-4mm of the apex adjacent to master cone was chosen and inserted as deep as possible into the canal. After removal, #25 finger spreader was placed adjacent to the heated master cone, removed immediately and replaced with an accessory cone with 2% taper. This process was repeated until complete obturation. Time required for obturation of each canal was also calculated from the time of placement of the first gutta-percha to completion of obturation using a chronometer (reported in minutes). A control radiograph was also obtained from the mesiodistal direction to ensure complete obturation.

The teeth were incubated at 37°C and 100% moisture for 14 days for completion of setting of sealer. Using an endodontic motor, root surface was marked at 3, 6 and 9 mm from the apex and the teeth were fixed to IsoMet cutting machine (Buehler IsoMet Low Speed Saw 11-1180) from the coronal part using glue. The roots were sectioned horizontally under copious water. The slices were immersed in sodium hypochlorite solution for one minute to eliminate dentin chips and were then rinsed with 17% EDTA (Calasept Nordiska Dental) for one minute followed by a rinse with water. The slices were photographed at ×40 magnification under a stereomicroscope (SZX9; Olympus, Japan).

Photographs were saved in TIF format and assessed in Adobe Photoshop CS6 1301.2 software. In this software, the total surface area of the canal in each section and the percentage of gutta-percha filled area (PGFA) and percentage of sealer-filled area (PSFA) were separately calculated on each image in pixels. The percentage of void areas [total area of section-(PSFA+PGFA)] was also calculated. These values were reported for each section. All measurements were made by an examiner blinded to the group allocation of teeth (Figure 1). The values were recorded in Microsoft Excel 2013.

The PGFA and PSFA data in different sections were analyzed using repeated measures ANOVA. Obturation time data were analyzed using ANOVA and data of percentage of voids were analyzed using the Kruskal Wallis test. The mean and standard deviation of PGFA, PSFA and void area were reported. For the comparison of the mean PGFA and PSFA, repeated measures ANOVA was used (since data were normally distributed). The Kruskal Wallis test was used to compare the mean area of voids in different sections. Data were analyzed using SPSS version 21 (SPSS Inc., IL, USA).

RESULTS AND DISCUSSION

Table 1 shows the mean PSFA, PGFA and void area values in apical, middle and coronal sections. Table 2 shows the mean time of obturation in the groups. Table 3 shows the P values for the comparisons. As seen in Table 3, in the apical section, CLNS group showed significantly lower PGFA than other groups ($P<0.05$). PGFA of WLS group was significantly higher than that of other groups followed by CLS, control and CLNS groups, respectively. The difference of WLS with CLNS was significant ($P<0.05$) but its difference with other groups was not significant ($P>0.05$) in this respect. The CLS group showed higher PGFA than the control group but this difference was not significant ($P>0.05$).

In the middle section, WLS showed higher PGFA than other groups followed by CLS, control and CLNS groups, respectively but only the difference between WLS and CLNS was significant ($P<0.05$). The CLNS group showed lower PGFA than other groups but this difference only for WLS was significant ($P<0.05$). The CLS showed higher PGFA than the control group but this difference was not significant ($P>0.05$).In the coronal section, the CLNS group showed significantly lower PGFA than other groups ($P<0.0001$). The PGFA in WLS group was higher than that in other groups followed by CLS, control and CLNS groups. The difference between CLNS and WLS was significant ($P<0.0001$) but other differences were not significant ($P>0.05$). The CLS group showed higher PGFA than the control group but this difference was not significant ($P>0.05$).

As seen in Table 4, in the apical section, the CLNS group showed significantly higher PSFA than other groups ($P<0.05$). The PSFA in WLS group was lower than that in other groups followed by CLS, control and CLNS groups, respectively. The difference between WLS and CLNS was significant ($P<0.0001$) but other differences were not statistically significant ($P>0.05$). The CLS group showed lower PSFA than the control group but this difference was not significant ($P>0.05$). In the middle section, WLS group showed lower PSFA than other groups followed by CLS, control and CLNS groups, respectively. Only the difference between the WLS and CLNS groups was significant ($P<0.05$). The CLNS group showed higher PSFA than other groups but this difference only with WLS was significant ($P<0.05$). The CLS group showed lower PSFA than the control group but this difference was not significant ($P>0.05$).

In the coronal section, the CLNS group showed significantly higher PSFA than other groups ($P<0.0001$). PSFA of WLS group was lower than that of other groups followed by CLS, control and CLNS groups, respectively. The difference of WLS and CLNS was significant ($P<0.0001$) but the difference with other groups was not statistically significant ($P>0.05$). In general, in all sections, the WLS group showed higher PGFA and lower PSFA than other groups followed by CLS, control and CLNS groups, respectively. The difference between WLS and CLNS in terms of PGFA and PSFA only in the middle third was statistically significant ($P<0.05$). The CLNS group in all sections showed lower PGFA and higher PSFA than other groups and this difference in the apical and coronal section was statistically significant. The CLS group compared to the control group showed higher PGFA and lower PSFA in all sections but these differences were not statistically significant ($P>0.05$).

No significant difference was noted in apical, middle and coronal sections of the four groups in terms of voids ($P>0.05$).

As seen in Table 5, the mean time required for root canal obturation in WLS group was significantly longer than that in other groups ($P<0.0001$) followed by the control, CLS and CLNS groups, respectively. The mean time required for root canal obturation in CLNS group was significantly shorter than that in the control group ($P<0.05$).

Three-dimensional obturation of root canal system is necessary to prevent bacterial reentry and ensure long-term success of endodontic treatment (Raina et al. 2007; Michaud et al. 2008). Finding an efficient technique with the greatest ability to provide a hermetic seal is important to guarantee the success of endodontic treatment (Haikei et al. 2006). This study compared the quality of obturation of straight root canals prepared with Mtwo rotary files and filled with four obturation techniques by measuring the surface area filled with gutta-percha and

Table 1. Mean PSFA, PGFA and void area in the apical, middle and coronal sections of the four groups

		Mean (%)	Standard deviation	Maximum	Minimum	Mean (%)	Standard deviation	Maximum	Minimum	Mean (%)	Standard deviation	Maximum	Minimum
Apical	control	6.08	2.37	9.67	2.61	92.85	2.18	96.36	89.33	0.06	0.18	0.6	0
	CLS	6.09	2.25	9.62	2.56	93.90	2.25	97.42	90.39	0	0	0	0
	CLNS	16.79	9.92	20.31	13.26	83.20	9.92	79.69	86.72	0	0	0	0
	WLS	5.03	3.44	8.55	1.50	94.96	3.44	98.48	91.45	0	0	0	0
Middle	control	4.43	2.33	7.07	2.73	95.09	2.33	97.27	92.91	0.47	0.84	2.20	0
	CLS	3.96	3.17	6.36	2.02	95.80	3.17	97.98	93.62	0.50	1.59	5.05	0
	CLNS	7.78	4.98	10.42	6.07	91.75	5.01	93.92	89.57	0.46	1.45	4.60	0
	WLS	3.95	2.33	6.12	1.77	96.04	2.33	98.22	93.86	0	0	0	0
Coronal	control	5.29	4.69	9.20	2.23	94.28	4.69	97.75	90.81	0.42	0.90	2.50	0
	CLS	5.23	3.59	9.11	2.15	94.46	3.48	97.93	90.99	0.40	0.81	2.50	0
	CLNS	19.2	8.54	24.13	17.16	79.35	8.54	82.82	75.88	1.44	1.84	4.56	0
	WLS	4.39	3.14	8.21	1.24	95.26	3.14	98.73	91.79	0.34	0.83	2.68	0

Table 2. Obturation time in the four groups

Group	Mean (minutes)	Standard deviation	Maximum	Minimum
Control	6.41	0.60	7.60	5.20
CLS	5.66	0.82	6.90	4.50
CLNS	5.03	0.82	6.20	3.90
WLS	8.43	1.18	10.30	6.50

that root canals filled with gutta-percha of Mtwo rotary system had higher quality of obturation compared to those filled with gutta-percha of Reciproc and WaveOne systems. Also, we wanted to compare the percentage of area filled with gutta-percha in case of using gutta-percha with 4% taper. Thus, Mtwo was a suitable choice for this purpose since #35 gutta-percha in Mtwo system has 4% taper. Although master cone in our study was #35 with 4% taper (matched single cone of Mtwo system), not all the prepared canals follow the shape and

Table 3. P values for the comparison of PGFA in the apical, middle and coronal sections of the four groups

	WLS			CLNS			CLS			Control		
	Coronal	Middle	apical	coronal	Middle	apical	coronal	middle	apical	Coronal	Middle	Apical
Control	1	1	1	<0.0001*	0.203	0.001*	1	1	1			
CLS	1	1	1	<0.0001*	0.067	0.001*				1	1	1
CLNS	<0.0001*	0.045*	<0.0001*				<0.0001*	0.067	0.001*	<0.0001*	0.203	0.001*
WLS				<0.0001*	0.045*	<0.0001*	1	1	1	1	1	1

*Significant.

Table 4. P values for the comparison of PSFA in the apical, middle and coronal sections of the four groups

	WLS			CLNS			CLS			Control		
	Coronal	middle	apical	coronal	Middle	apical	coronal	middle	apical	Coronal	Middle	Apical
control	1	1	1	<0.0001*	0.206	0.002*	1	1	1			
CLS	1	1	1	<0.0001*	0.068	0.001*				1	1	1
CLNS	<0.0001*	0.046*	<0.0001*				<0.0001*	0.068	0.001*	<0.0001*	0.206	0.002*
WLS				<0.0001*	0.046*	<0.0001*	1	1	1	1	1	1

*Significant.

sealer using area-metric analysis. The results showed that root canal obturation with non-standard accessory cones and a master cone with 4% taper decreased the gutta-percha mass and increased the sealer area. Use of a master cone with 4% taper had no positive effect on quality of obturation or working time. Warm lateral compaction in straight canals had no superiority over cold lateral compaction and took longer to accomplish.

In our study, root canals were prepared using Mtwo rotary system since Schafer et al. (2016) in 2016 showed

Table 5. P values for the comparison of obturation time in the four groups

Group	WLS	CLNS	CLS	Control
Control	<0.0001	0.01	0.28	-
CLS	<0.0001	0.43	-	0.28
CLNS	<0.0001	-	0.43	0.01
WLS	-	<0.0001	<0.0001	<0.0001

taper of final file after preparation with the final rotary file, so we designed a group in which we placed accessory gutta-percha cones between single cone and canal walls to assess the possibility of improving the quality of obturation. Apical seal is an important parameter in success of endodontic treatment and improper apical seal results in development of periapical lesions. The results of our study showed that in the apical third, use of non-standard gutta-percha alongside the master cone with 4% taper significantly decreased the apical seal and quality of obturation and decreased PGFA and increased PSFA. Also, our results showed that use of standard gutta-percha with 2% and 4% taper along with 2% accessory cones did not cause a significant change in quality of obturation in the apical third. Although PGFA in 4% gutta-percha group was higher and PSFA was lower than that in other groups, these differences were not significant and no difference in voids was noted either. Based on our results, warm lateral com-

paction had no superiority over cold lateral compaction since no difference was noted between the two groups in any of the assessed parameters.

Comparison of the mean time of obturation among the groups showed that the mean time of obturation was significantly longer in WLS group compared to other groups. This time in CLNS group was significantly shorter than that in the control group. The CLS and control groups were not significantly different in this regard. Schafer et al. in 2012 compared the quality of root canal obturation with 2% and 4% taper gutta-percha cones by microscopic area-metric analysis and found no significant difference between the two types in the apical region. None of them provided a better apical seal. Romina et al.(2009) performed area-metric analysis of root filling and showed equal apical seal in use of gutta-percha cones with 2% and 4% taper. Our results were in agreement with those of the afore-mentioned studies.

We found no significant difference in time of obturation between the use of gutta-percha cones with 2% and 4% taper, which was in agreement with the results of Schafer et al.(2012). Our results regarding the use of non-standard MF gutta-percha cones in comparison with the use of standard gutta-percha showed that use of non-standard cones along with a master cone with 4% taper decreased PGFA and increased PSFA in all sections and this difference in the apical and coronal thirds was significant. Thus, use of MF accessory cones along with 4% gutta-percha does not provide suitable apical seal. In contrast to our results, VanGheluwe and Wilcox in 1996 found no significant difference in apical seal of standard and non-standard gutta-percha cones. Difference in the results of the two studies may be due to the use of curved roots in their study. Use of warm lateral compaction method in our study increased PGFA and decreased PSFA in all sections, although insignificantly. No difference was noted in terms of voids. No significant difference between warm and cold lateral compaction techniques in our study may be due to our small sample size and use of straight canals because cold lateral compaction in straight canals is much easier and yields better results than in curved canals.

Thus, future studies with larger sample sizes are required to compare these two methods in curved canals. Collins et al. (2006) compared warm and cold lateral compaction and warm vertical condensation and concluded that warm lateral compaction significantly increases the volume of gutta-percha mass in the canal compared to cold lateral compaction. Difference between our results and theirs may be due to different methodology since we performed area-metric analysis while they used replication of canal irregularity. Moreover, they used D11T spreader in their study, which is very fine and made of stainless steel, and can penetrate to 1mm of the working length while we used finger spreader penetrated to 2mm of the working length. Moreover, they used non-standard MF gutta-percha while we used standard cones with 2% taper.

Several methods are available for assessment of the quality of root canal filling such as dye penetration (McRobert et al. 997), microbial leakage models, replication of canal irregularity (Jacobson et al. 2008) and three-dimensional analysis by micro-computed tomography (CT) (Hammad et al. 2009). These methods have advantages and limitations. Dye penetration and microbial leakage are more commonly used due to simplicity and acceptable accuracy; however, smaller size of dye particles than some bacteria (Nielsen et al. 1995) and complexity and time consuming nature of microbial leakage (Sequeira et al. 2000) are among the drawbacks of these techniques.

Area-metric analysis has been used by many researchers for assessment of the success of endodontic treatment in vitro. Thus, this method was used in our study. The limitations of this method include two-dimensional analysis of the root canal filling and lower accuracy than micro-CT. Moreover, dentin chips and smear layer created during sectioning in this method may decrease the quality of images and cause erroneous determination of borders between gutta-percha, sealer and voids. To overcome this limitation, we immersed the slices in sodium hypochlorite solution for one minute after sectioning followed by one minute in 17% EDTA and then rinsed them to eliminate the smear layer and dentin chips. Considering the higher accuracy of micro-CT for three-dimensional assessment of quality of obturation, further studies with the use of micro-CT on both straight and curved canals are required to obtain more accurate information on this topic.

CONCLUSION

Root canal obturation with non-standard accessory cones and a master cone with 4% taper decreases the gutta-percha mass and increases the sealer area and therefore, compromises the quality of root canal filling. Use of a master cone with 4% taper has no positive effect on quality of obturation or working time. Warm lateral compaction in straight canals has no superiority over cold lateral compaction and takes longer to accomplish.

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Correlation of the maxillary sinus volume with gender and some of craniofacial indices using cone beam computed tomography

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ABSTRACT

Maxillary sinus volume is variable and thus, is a topic of interest for surgeons performing endoscopic surgeries. Also, it may be helpful for sexual identification. This study sought to measure the maxillary sinus volume using cone beam computed tomography (CBCT) and assess its correlation with gender and some craniofacial indices. In this descriptive, analytical, cross-sectional study, CBCT scans of 70 patients (35 males and 35 females) older than 18 years of age were evaluated. To measure the maxillary sinus volume, axial CBCT sections with 2mm slice thickness and Digimizer software were used. Sinus volume was calculated using the formula: volume=sum of the thickness of each slice multiplied by the surface area of each slice. The mean width and height of the palate in the coronal plane, anterior-posterior length of the palate in the sagittal plane and distance between the two zygomatic buttresses were measured on the axial CBCT sections in both males and females. The Pearson's correlation coefficient, independent t-test and paired t-test were used for statistical analyses. The mean maxillary sinus volume was 15.9±6.05 cm3 and 13±2.85 cm3 in males and females, respectively. The mean volume of the maxillary sinus was larger in males, and a significant correlation was noted between the mean volume of the maxillary sinus and width and height of the palate as well as the distance between the two zygomatic buttresses ($P<0.05$). Despite the larger volume of the maxillary sinus in males, this parameter cannot be used for sexual identification because the area under the receiver operating curve (ROC) was 62.7%.

KEY WORDS: CONE-BEAM COMPUTED TOMOGRAPHY; SEXUAL IDENTIFICATION; MAXILLARY SINUS

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INTRODUCTION

Maxillary sinus is an irregular cavity within the maxillary bone, which develops under the orbit. Development of the maxillary sinus starts in the third week of pregnancy and continues to the pubertal period (Wang et al. 1994). Accurate measurement of the maxillary sinus volume may be helpful prior to sinus floor augmentation for implant placement (Gray et al.2000). Maxillary sinus volume is variable and thus, is a topic of interest for surgeons particularly the oral and maxillofacial surgeons (Fernandes, 2004). Recently, demand for sexual identification in forensic medicine has increased due to an increase in rate of crime (Saccucci et al.2015). The correlation of volumetric analyses of the maxillary sinus and sexual identification has been a controversial topic (Ariji et al. 1994; Jasim et al. 2013; Vidya et al. 2013; Ekizoglu et al.2014; Möhlhenrich et al. 2015).

Conventional radiographs are not accurate for determination of the volume of the maxillary sinus due to the superimposition of images of other paranasal sinuses and craniofacial skeleton on the superior part of the maxillary sinuses (Wolf et al.1993). Considering the complex structure of the sinus, magnetic resonance imaging and computed tomography (CT; gold standard) are used for three-dimensional visualization of the sinus anatomy. However, their use is limited due to high patient radiation dose (CT), limited access and high cost (Schwab et al.1998; Lenza et al.2010). Thus, this study aimed to measure the volume of the maxillary sinus using cone beam computed tomography (CBCT) and assess its association with gender and some craniofacial indices.

MATERIAL AND METHODS

This study was conducted on CBCT scans of the right and left maxillary sinuses of 70 patients including 35 females and 35 males over 18 years of age, who presented to the Oral and Maxillofacial Radiology Department of Islamic Azad University, Isfahan branch. Images with optimal quality were chosen. All images had been taken with the same CBCT unit (Galileos-Sirona, Bensheim, Germany) with high resolution and 0.1 mm voxel size and exposure settings of 85 kV and 21-35 mAs (based on the patient's weight) and were analyzed using SIDEXIS software. Patients with a history of surgery or facial asymmetry, completely or partially edentulous patients, those with inflammatory diseases of the sinus such as sinusitis, periodontal patients, those with tumors such as angioma, squamous cell carcinoma and lymphoma, history of facial bone fracture, cysts, infection and sinus polyps were excluded.

Distance between the zygomatic buttresses: Distance between the zygomatic buttresses was calculated on axial sections of the upper midface according to a study by Ariji et al. [9] and Waitzman et al.(1992). To calculate this distance, on axial sections visualizing the inferior border of the zygomaticomaxillary suture, a horizontal line was drawn connecting the outermost points of the zygomatic buttresses in the two sides and its length was measured in millimeters (Figure 1a).

Width and height of the palate: To calculate the palatal width on coronal sections according to Byasalet al. (2011), a horizontal line was drawn from the palatal margin of the crestal bone of the first molar tooth in one side to the palatal margin of the crestal bone of the other side and its length was measured. To measure the height of the palate in this region on coronal sections, as shown in Figure 1b, a line was drawn from the palate perpendicular to the line connecting the alveolar crest borders of the two sides and its length was measured.

Length of the hard palate: To measure the length of the hard palate on sagittal sections, the distance between the anterior nasal spine and posterior nasal spine at the midline (where the anterior nasal spine was visible) was measured (Figure 3C).

Maxillary sinus volume: To measure the maxillary sinus volume, axial CBCT sections with 2mm slice thickness and 2mm intervals were used. The CBCT sections were subjected to Digimizer version 4.1.1 software and the surface area of the sinus was automatically measured and stored in Excel format. The surface area of all sections of the sinus (about 20 to 25 sections) in both sides was calculated for each patient. Then, to calculate the volume of the sinus in cubic centimeters, the following formula was used: Sum of the surface area of the sinus in each section × thickness of each section (Figure 2). Data were collected and analyzed using SPSS version 24.Independentt-test and paired t-test were used to compare differences in the right and left sides and in the mean size of variables.

RESULTS AND DISCUSSION

Two groups (n=35) of males and females in the age range of 18 to 74 years were evaluated. The mean age was 37.2 years in females and 34.3 years in males. Independent t-test showed that the mean age of males and females was not significantly different (P=0.30). The mean volume of the maxillary sinus was 14.45±2.71 cm^3. Independent t-test showed that the mean volume of the sinus in the right (P=0.02), left (P=0.006) and both (P=0.01) sides was significantly greater in males than females. In other words, a significant association existed between gender and maxillary sinus volume in the right and left sides (P<0.05, Table 1).

The area under the ROC curve was 0.627 or 62.7% (smaller than 0.70) (Graph 1).

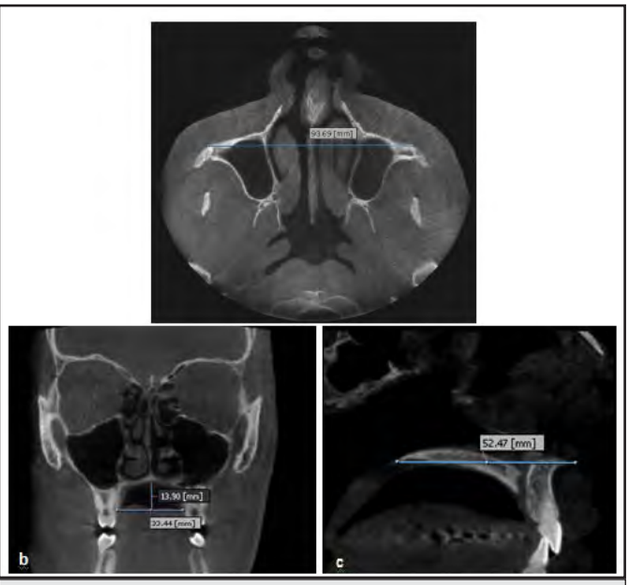


FIGURE 1. (a) Distance between the two zygomatic buttresses. (b) Measurement of the width and height of the palate at the maxillary first molar site. (C) Measurement of the anterior-posterior length of the hard palate

Paired t-test showed that the mean volume of the maxillary sinus in the right and left sides in females (P=0.61), males (P=0.68) and in total (P=0.92) was not significantly different (Table 2).

Independent t-test showed that the mean anterior-posterior length of the palate in sagittal plane was not significantly different between males and females (P=0.23) but the mean distance between the two zygomatic buttresses in the axial plane (P<0.001), palatal width in the first molar region in the coronal plane (P=0.007) and height of the palate in the first molar region in the coronal plane (P=0.002) were significantly greater in males than in females. Thus, no significant association existed between gender and anterior-posterior length of the palate in sagittal plane; but gender had a significant association with the other three variables (Table 3).

The Pearson's correlation coefficient showed that no significant association existed between age and vol-

Table 1. Comparison of the mean volume of the maxillary sinus between males and females in the right and left sides (in cm3)

Side	Females		Males		Independent t-test	
	Mean	Standard deviation	Mean	Standard deviation	t	P value
Right	13/1	2/9	15/8	6/3	2/31	0/02
Left	12/9	2/8	16/01	5/8	2/86	0/006
Total	13	2/85	15/9	6/05	2/58	0/01

Table 2. Comparison of the mean volume of the maxillary sinus in the right and left sides based on gender (in cm3)

Gender	Right		Left		Independent t-test	
	Mean	Standard deviation	Mean	Standard deviation	t	P value
Females	13/1	2/9	12/9	2/8	0/51	0/61
Males	15/8	6/3	16/01	5/8	0/41	0/68
Total	14/4	55/1	14/5	4/8	0/09	0/92

Table 3. Comparison of the mean craniofacial parameters in males and females (in mm)

Parameter	Females		Males		Independent t-test	
	Mean	Standard deviation	Mean	Standard deviation	t	P value
Distance between zygomatic buttresses in axial plane	85/2	4/2	88/9	4/1	3/70	<0/001
Anterior posterior length of the palate in sagittal plane	51/2	3/2	52/6	6/1	1/20	0/23
Width of the palate in first molar site in coronal plane	33/2	2/6	34/9	2/8	2/79	0/007
Height of the palate in the first molar site in coronal plane	11/8	1/7	13/8	3/3	3/25	0/002

Table 4. Pearson's correlation coefficient for the correlation between the mean size of the maxillary sinus and age and craniofacial parameters

Variable	Maxillary sinus volume	
	r	P value
Age	-0/182	0/18
Distance between zygomatic buttresses in axial plane	0/424	<0/001
Anterior posterior length of the palate in sagittal plane	0/119	0/33
Width of the palate in first molar site in coronal plane	0/241	0/044
Height of the palate in the first molar site in coronal plane	0/547	<0/001

ume of the maxillary sinus (P=0.18). The anterior-posterior length of the palate in the sagittal plane had no significant association with the maxillary sinus volume (P=0.33). But an association existed between the palatal width in the first molar site in the coronal plane and the volume of the maxillary sinus (P=0.044). The Pearson's correlation coefficient showed that a significant association existed between the distance between the two zygomatic buttresses in the axial plane and also the height of the palate in the first molar region in the coronal plane with the volume of the maxillary sinus (P<0.001, Table 4).

Maxillary sinus is the closest paranasal sinus to the oral cavity and since implant treatments along with open or closed sinus lift surgery (due to pneumatization of the maxillary sinus) have greatly increased, knowledge about the anatomy of the maxillary sinus is impor-

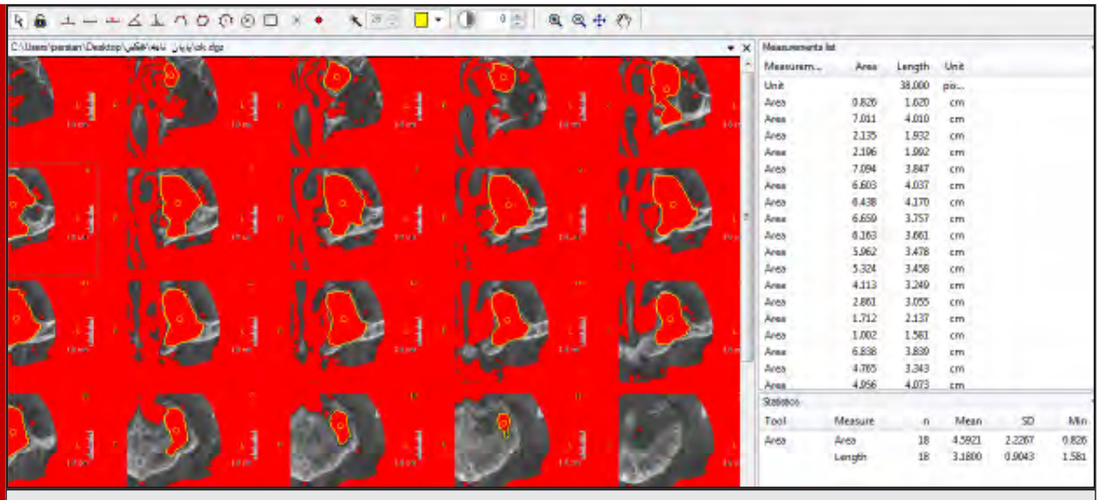
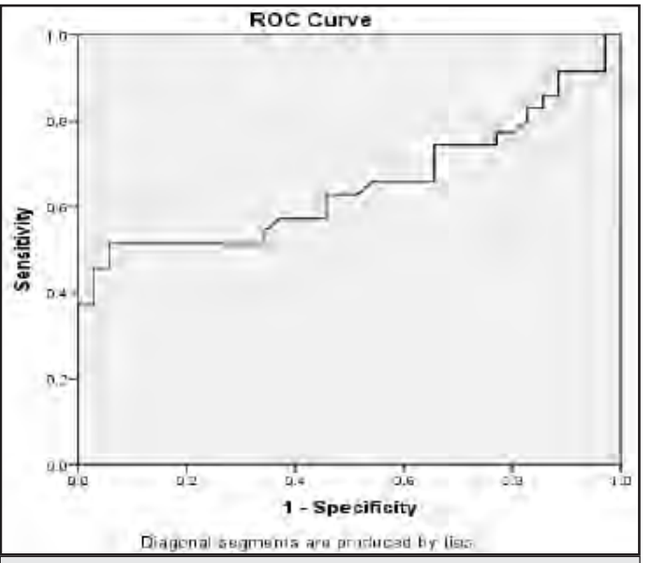


FIGURE 2. Method of measurement of maxillary sinus surface area using Digimizer software



GRAPH 1. ROC curve for sexual identification using maxillary sinus volume in the right and left sides

tant (Gray et al. 2000). Sexual identification is an important parameter in forensic medicine. Use of maxillary sinus dimensions measured on CT scans may help in this regard in cases where other methods fall short; however, this method has its own drawbacks as well (Lerno, 1983). Since a decade ago, CBCT is recommended as a modality for maxillofacial imaging (Farman et al. 2006; Moreira et al. 2009). This modality enables image reconstruction without superimposition and has lower patient radiation dose compared to CT (Moreira et al. 2009). It is believed that the quality of CBCT images is equal to that of conventional CT in visualization of maxillofacial structures (Silva et al. 2008; Loubele et al. 2009; Carrafiello et al. 2010).

In our study, the mean volume of the right maxillary sinus was 14.4±5.1 cm^3 and the mean volume of the left maxillary sinus was 14.5±4.8 cm^3. Also, maxillary sinus volume in males was significantly greater than that in females ($P<0.05$). In 2013, Jasim et al. measured the volume and dimensions of the maxillary sinus and evaluated its association with age and gender using CT. They reported that the volume and dimensions of the maxillary sinus in males were greater than that in females and volume of the sinus decreased by an increase in age (Jasim et al. 2013). To calculate the volume of the maxillary sinus, they used a method similar to ours; however, in our study in contrast to theirs, no significant association was found between age and volume of the maxillary sinus ($P=0.30$). This difference may be due to the age group of patients since Jasim et al. (2013) evaluated patients in older age group (40-69 years).

In 2016, Prabhat et al. evaluated the size and volume of the maxillary sinus and its association with sexual identification using CT. They reported that volume of the maxillary sinus in males was significantly greater than that in females. They stated that use of maxillary sinus volume increases the accuracy of sexual identification by 83.3% (Prabhat et al. 2016). In our study, volume of the maxillary sinus in males was greater than that in females. However, since the area under the ROC curve was 62.7% (<70%), maxillary sinus volume cannot be used as a reliable indicator for sexual identification. These differences in measurements may be due to anatomical differences of the sinus in different geographical areas and among different races. Another reason may be the difference in method of calculation of sinus volume and sample size, since our sample size was twice as big as that of Prabhat et al.(2016).

Senturk et al. in 2015 evaluated the effect of weather and altitude on volume of the paranasal sinuses and found no significant association between the volume of the maxillary sinus and age or between the size of the sinus in the two sides (Sahlstrand-Johnson, 2011). In our study, no significant difference was noted between the volume of the maxillary sinus and age or between the volume of the maxillary sinuses in the two sides ($P>0.05$). Ariji et al. in 1993 evaluated the correlation of the maxillary sinus volume and aging and found that the mean volume of the right and left maxillary sinuses was 14.64±6.32 cm^3. They found no significant difference in the volume of the maxillary sinus between males and females. Sinus volume had an ascending trend to

20 years of age and then decreased. They also reported that a significant association exists between the volume of the maxillary sinuses and the distance between the two zygomatic buttresses and the zygomatic occipital distance (Ariji et al. 1994). The maxillary sinus volume reported by Arihi et al. (1994) was comparable to the value obtained in our study (14.45±2.71 cm^3).

However, in our study, the volume of the maxillary sinus in males was significantly higher than that in females and no significant association was noted between the sinus volume and age. In our study, aside from the relationship of the maxillary sinus volume with age and sex, its correlation with some other craniofacial parameters such as width and height of the palate in the first molar area in the coronal plane, anterior-posterior length of the palate in the sagittal plane and the distance between the two zygomatic buttresses in the axial plane was also evaluated and it was found that a correlation existed between the volume of the maxillary sinus and the distance between the two zygomatic buttresses; this finding was comparable to that of Ariji et al. Moreover, it was found that the greater the width and height of the palate, the greater the size of the maxillary sinus would be ($P<0.05$). But no significant correlation was found between the sinus volume and the anterior-posterior length of the hard palate ($P>0.05$).

CONCLUSION

The current results showed that Despite the presence of a significant association between gender and volume of the maxillary sinus ($P<0.05$), since the area under the ROC curve was 0.627 or 62.7% (smaller than 0.70), volume of the maxillary sinus cannot serve as a definite and reliable indicator for sexual. Also, a correlation was noted between the maxillary sinus volume and width and height of the palate at the first molar site and the distance between the two zygomatic buttresses.

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Comparison of interleukin 17 and 22 in saliva of oral lichen planus patients with healthy people

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ABSTRACT

Lichen Planus (LP) is an inflammatory chronic mucocutaneous disease. Since the etiology of OLP is unknown, efforts to understand the etiology and pathogenecity may lead to improve treatment modalities. Studies have shown the different levels of increase or decrease of various cytokines in saliva, serum or tissue culture of patients with OLP.As saliva originates from blood and its collection is an easy, non-invasive method, so it can be a diagnostic fluid that has logistical advantages when compared with serum. The purpose of this study was to compare the salivary level of IL17 and IL22 in patients with and without OLP. Saliva of 52 patients with and without OLP was collected. The saliva level of IL17 and IL 22 was measured by using ELISA. Data were analyzed using independent T test, Mann-Whitney and Pearson Correlation test.The saliva concentration of IL 22 was significantly higher in control group than OLP but the difference was not significant between reticular and atrophic-erosive type ($P > 0.05$).The difference between mean rank of IL17 for OLP and healthy cases and between reticular and erosive-atrophic form was not significant ($P > 0.05$). There was no correlation between the level of IL17 and IL 22 ($P > 0.05$). Our findings suggest that IL 22 may have an effect in the pathogenesis of OLP and its protective effect may be decreased in OLP patients.

KEY WORDS: INTERLEUKIN 17, INTERLEUKIN 22, ORAL LICHEN PLANUS, SALIVA

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INTRODUCTION

Lichen Planus (LP) is an inflammatory chronic mucocutaneous disease that affects 1-2% of the general population, and is the non-infected mucosal disease causing the most referral to dental clinics (Pakfetrat et al. 2009). The oral cavity is the most common site of the disease. Oral Lichen Planus (OLP) usually affects middle aged women (30-60 years), children are rarely affected.World Health Organization (WHO) has considered OLP as a pre-cancerous disorder. Although OLP is considered an immunologically mediated disease, its etiology is not known, yet. The involvement of both specific and non-specific antigens are considered to have roles in pathogenesis of OLP (van der Meij et al. 2003 Aghahosseini et al. 2006; Pourshahidi et al. 2011; Agha-Hosseini et al. 2015).

Alteration of the immune condition is not well known in OLP to date, but it is clear that cytokines exhibit immunoregulatory actions through a complex cytokine network consisting of paracrine and autocrine systems. Unbalanced cytokine actions are considered to play a role in the pathogenesis of autoimmune diseases. Numerous cytokines are secreted from oral mucosa or blood as locally or systemically that they have an important role in initiation and progression (courses) of OLP (Zhang et al. 2008).

Increased production of cytokine by keratinocytes that may be the result of stimulation by external stimulants is the initial event. Then infiltration of monocytes followed by lymphocytes could be the next step. Studies have shown the different levels of increase or decrease of various cytokines in saliva or serum or tissue culture of patients with OLP (Yamamoto et al. 1990; Rhodus et al. 2005; Zhang et al. 2008).

Interleukin (IL) 17 A, also named IL17, is the most important IL from the IL17 family which is composed of 6 members (IL17A-IL17F). IL 22 is a member of IL10 family and it has a role in immune reaction against bacterial pathogens, especially in epithelium. IL17 and 22 are produced by T helper 17 and contribute in both innate and acquired immune responses against extra cellular pathogens (Lambiase et al. 2009). The role of Th17 and its cytokines (IL17 and 22) have been established in inflamed tissues in various autoimmune diseases such as psoriasis, multiple sclerosis, lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease (IBD) and pemphigus (Miossec et al. 2009).Saliva originates from blood. Saliva collection is an easy, non-invasive method, and it has been shown that various tumor markers are present in saliva. Therefore it can be a diagnostic fluid that has logistical advantages when compared with serum (Agha-Hosseini et al. 2015). The purpose of this study was to compare the level of IL17 and IL22 in saliva in patients with and without OLP.

MATERIAL AND METHODS

Fifty two samples of referred patients to the Department of Oral Medicine, Faculty of Dentistry, Tehran University of Medical Sciences included in this cross-sectional study (26 in each group).this study was approved by ethical committee and also all subjects provided written informed consent to participate in the study. OLP was diagnosed clinically and confirmed histopathologically according to the modified WHO criteria in 2003 as below:Clinical criteria: presence of symmetric or bilateral reticular or papular lesions with or without erosive–atrophic components. Histological criteria: presence of well-defined band-like zones of inflammatory infiltration confined to the superficial part of connective tissue, consisting mainly of mature lymphocytes; signs of "liquefaction degeneration" in basal cell layer, absence of epithelial dysplasia. Exclusion criteria: application of any local treatment for OLP lesions during the previous month; taking any drugs during the previous 3 months; history of allergy to foods or environmental factors, smoking; the existence of any oral lesions except OLP, systemic diseases (cardiovascular disease, kidney, hypertension), pregnancy. We also excluded patients whose lesion was nearby amalgam restorations. The unstimulated whole saliva (UWS) was collected between 10:00 a.m. and 12:00 p.m., and at least 90 minutes after the last intake of drink or food. All subjects were requested to swallow first, tilt the head forward and then expectorate at least 5cc UWS into a sterile centrifugal tube without swallowing. The samples were centrifuged (2000g for 10 min), and the supernatants stored at -20 oC until analysis. The saliva level of IL was measured by using Enzyme-Linked Immunosorbent Assay (ELISA) kits, Human platiniumIL17A (BMS2017) and Human plutonium IL22 (88-7522-22) (bioscience, USA).

STATISTICAL ANALYSIS

Independent T test was used to compare the mean of IL22 between two groups; and Mann-Whitney for IL17. Pearson Correlation test was used for assessing the correlation between IL 17 and IL22 in each group. A P value less than 0.05 was considered statistically significant and primary power of analysis was set at 80%.

RESULTS AND DISCUSSION

26 patients with OLP (22 females and 4 males) and 26 sex and aged matched healthy individuals were enrolled in this study. The mean age was 50.81±12.61 years, and 49.42±5.33 years in OLP and control groups respectively. 23% (6 patients) of OLP was reticular form, and 77% (20 patients) was atrophic-erosive form. The mean

of IL22 was 184.48±4.58 pg/ml in OLP and 209.01±4.58 pg/ml in control group, there was significant difference between two groups (p=0.003). The mean of IL 22 was 176.5±9.40 pg/ml in reticular and 186.88±5.26 in atrophic-erosive type but the difference was not significant (p=0.35).

The mean of IL 17 was 5.24±2.709 pg/ml in OLP and 0.526±0.164 pg/ml in control. The mean rank of IL17 was 37.33 and 33.59 for OLP and healthy cases respectively. The difference was not significant (p=0.436). The difference between the mean of IL17 in reticular and erosive-atrophic form was not significant (p=0.25).

There was no correlation between the level of IL17 and IL22 (p=0.35, r=-0.19)in healthy patients. Although correlation analysis revealed no significant correlation between IL17 and IL 22 in OLP group but it seems to be a reverse correlation between them according to the statistical findings (p=0.054, r=- 0.049).

As the etiology of OLP is unknown and because it is one of the most common lesion referred to dental clinics, studies on the pathogenesis of the OLP are of high importance. In this study we evaluated the relevance of IL17 and IL22 with OLP. The saliva concentration of Interleukin 22 was significantly higher in control group than OLP but the difference was not significant between reticular and atrophic- erosive type (P>0.05). We couldn't find any studies on OLP patients' salivary levels of IL22. But Chen et al and Shen et al studied on IL22 in the tissue samples from OLP patients and found it more than their control group (Chen et al. 2011; Shen et al. 2012). More expression of IL22 in the tissue may be due to the location of its receptors which is on the epithelial origin cells (Ouyang et al. 2008). On the other hand there are some researches on the role of IL22 and psoriasis (Zheng et al. 2007; Lowes et al. 2008). Zaba et al. (2007) in an animal study reported the higher expression of IL17 and IL22 in skin lesions of mice with psoriasis; but Zheng and Ma reported that deficiency and limitation of IL22 can exaggerate skin lesion of psoriasis in mice (Zaba et al. 2007; Zheng et al. 2007; Ma et al. 2008). This controversy reveals that more studies should be done on the subject. The salivary level of IL17 in OLP patients of our study was higher than the control group but the difference was not statistically significant (p=0.436). On the basis of our knowledge there is no study on salivary level of IL17 in OLP.

Zhang et al. (2007) compared the level of IL1, IL8 and TNFα in serum and saliva in 30 OLP patients and 30 healthy controls. These IL were higher significantly in saliva and serum in patients than control, and the level of IL8 was higher in saliva in erosive OLP than other types (Zhang et al. 2008). Xie et al. (2012) assessed Th1 and Th17 in tissue (by double immunofluorescence staining) and in serum (by flow cytometry) of 40 patients with OLP (22 cases with reticular type and 18 cases with erosive- atrophic type). Their results showed that the Th17 and Th1 cells were similar in tissue, but both were increased in serum. Th17 was higher in erosive- atrophic OLP than reticular. The level of IL 17 was significantly higher in serum of OLP patients than controls and in erosive-atrophic OLP than reticular.

The role of IL17 has been assessed in other autoimmune diseases in previous studies (Moseley et al. 2003; Nistala et al. 2009; Caproni et al. 2009). Caporni and et al. (2009) evaluated the level of IL17 in serum of psoriasis patients before and after treatment, and compared with healthy controls. The level of IL17 was higher in patients than control and was reduced to a low level after treatment [21]. Ziolkowska et al measured the level of IL17 in serum and synovial fluid of joints in 15 patients with rheumatoid arthritis, and compared with healthy controls. Their results showed higher level of IL17 in patients than control (Ziolkowska et al. 2000). Zhao et al studied the role of Th17 in asthma sensitivity. Percentages of Th17 cells and the level of several cytokines including IL4, IL22, IL25, IL17 and INFγ were measured. Th17 cells and related cytokines had the most increasing; additionally, the amount of IL17 and IL22 and Th17 had a positive correlation with the severity of disease (Zhao et al. 2010).

A hypersensitivity reaction is considered in pathogenesis of OLP when cytokines are released by activated T cell, resulting in accumulation of inflammatory cells, and the destruction of keratinocytes due to the cytotoxicity of cells (Sezer et al. 2007).

The immune mechanism with specific and non specific antigen are proposed in the pathogenesis of OLP (Sugerman et al. 2005). IL17 may up regulate various cytokines like IL1, IL8 and TNFα (Hwang et al. 2004). In the study of Zhang et al (2008) the serum and salivary level of these cytokines was elevated in OLP patients such as other studies (Sun et al. 2005; Rhodus et al. 2006; Janardhanam et al. 2007; Rhodus et al. 2007). Therefore, it may be postulated that IL17 probably has a role in pathogenesis of OLP by increasing these inflammatory cytokines. In non specific antigen, another mechanism may be degranulation of mast cells and activation of MMP (Sugerman et al. 2005). Jovanovic et al (2001) reported that IL17 acts as an up regulator of local inflammatory factors, causing injury of extracellular matrix through MMP (in vitro). Therefore it may be an influence in pathogenesis of OLP. In present study the level of IL17 was ten times higher than the level of healthy control (5.24 pg/ml in patients and 0.516 pg/ml in healthy subjects), although the difference was not significant statistically.

It is clear that IL22 has protection effects while IL 17 has not (Zenewicz et al. 2007; Sonnenberg et al. 2010). Consequently, with the lower level of IL22 and higher

level of other inflammatory cytokines, epithelial cells are disposed to destroy, and OLP lesions can appear. This hypothesis of the reverse correlation between IL17 and IL22 in patients with OLP is strengthened, but it needs more research.

CONCLUSION

Our findings suggest that IL 22 may have an effect in the pathogenesis of OLP and its protective effect may be decreased in OLP patients.

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On the prevalence of iron deficiency in children and adolescents with growth retardation

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ABSTRACT

Iron is a fundamental micro-element in body. Iron deficiency is the most prevalent nutritional deficiency around world. Iron deficiency can disarrange physical growth and neurocognitive development of children thorough different ways such as decreasing oxygen transport capacity, decreasing energy production and decreasing the appetite. The aim of this study was to evaluate iron deficiency prevalence in children between 6-18months old in Babol, Iran. This cross sectional study was performed on 100 children and adolescents with growth retardation in Babol. Weight and height of children was measured and comparing with the growth curve indices, Hb and mid MCV of children in percentile Height and weight lesser than 3 was evaluated. In this study, prevalence of anemia in growth retarded children was 35.9% whereas prevalence of microcytic anemia was 20% followed by iron deficiency prevalence of 1.6% and 25%. Average of ferritin level decreased with increasing of severity of growth disorder in children. The results of this study suggest that treatment of growth retarded children with iron supplements can improve their growth. Because data about prevalence of IDA in Iranian children is limited, we suggest further studies to be performed to define an average range of iron concentrations in accordance with other micro-elements such as zinc and copper in children of this area.

KEY WORDS: GROWTH RETARDATION, CHILDREN AND ADOLESCENTS, IRON DEFICIENCY ANEMIA

INTRODUCTION

Iron deficiency is a worldwide problem during life, but infants are especially susceptible to development and complications of iron deficiency (Akramipour et al., 2008). Iron deficiency during first two years of a child life can cause irrecoverable deficits in cognitive development, and also other potential adverse effects(Black et al., 2013, Victora et al., 2008) 2013, Victora et al., 2008. Iron requirements of infants under six months old, are generally not well determined, since it is difficult to estimate the demands in infants which exclusively eat breast milk (Bender, 2003) Assumed that most of newborn infants are greatly protected from iron deficiency

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by the birth iron stores, usually iron deficiency is not considered in infants below six months old. However, iron deficiency (ID) and iron deficiency anemia (IDA) can be observed, in the range of 0%–15% (ID) and 0%–4% (IDA) in six-month-old infants around the world (Ziegler et al., 2009, Nguyen et al., 2017b).

Older infants (up to 24 months old) are at higher risk of ID and IDA than younger one. Infants are susceptible to the effects of low iron levels, even before the first moments of birth: low iron stores at birth have been associated with iron deficiency and also elevated risk of growth retardation and cognitive developmental disorders later in infancy (Ziegler et al., 2009, MacQueen et al., 2017).

Iron deficiency developing in infancy and leads to IDA has similarly been associated with bad cognitive, behavioral and growth problems; the consequences of iron deficiency without IDA are not clear (Kazal Jr, 2002 Domellöf et al., 2014, Mamabolo and Alberts, 2014, Ritu et al., 2017).

In Iran, there may be not an ordinary screening program for anemia and ID in children and population-based data on anemia, that includes ferritin concentration in children, continue to be limited. The aim of present study was to identify the prevalence of iron deficiency and explores their associations with socio-economic parameters and it's complications in growth failure in and adolescents of 1-18 years old in Babol, Iran.

MATERIAL AND METHODS

In this cross-sectional study blood samples were collected from 100 children and adolescents (56 boys and 44 girls). They aged 1 to 18 years with growth failure including small height, low weight or both of them whom referred to our Hospital during years 2015-2016 were investigated. The patients were divided to 3 categories according to the place of life (urban, suburb, and rural). 70 individuals were urban patients (%70), 24 rural patients (24%) and 6 suburb patients (6%).

The patient's weight and height were measured and compared according to growth curve indices (CDC) and patients with growth retardation (percentile of weight and height under 3%) were evaluated for iron deficiency associated hematologic factors (blood hemoglobin level, MCV, and serum ferritin level). Other factors such as birth weight and the location of life were also evaluated in the patients. The exclusion criteria were patients with any associated disease such as heritable problems and liver-heart or kidney diseases, and also patients under treatment with iron were omitted from the study. Children with growth disorder having serum ferritin levels lower than normal (according to normal value associated with age and sex) regardless to presenting anemia or not are considered as positive cases of the study.

STATISTICAL ANALYSIS

The Hb concentrations were symmetrically distributed. The distribution of plasma ferritin was skewed regarding higher values. The descriptive statistics included means, medians, interquartile ranges, and 95% assurance intervals as appropriate. Student t-test or Chi-square tests were used to measure the dissimilarity between Hb and ferritin with clinical or other baseline features. Data statistical analyses was performed via SPSS software version 18.

RESULTS AND DISCUSSION

Anemia in little children lower than 2 years of age is of ultimate concern since their fast growth needs a high iron consumption which can be regularly not covered by their food plan, (Kotecha, 2011, Nguyen et al., 2017a). It was not possible to estimate the anemia prevalence in this group separately due to the minimal records. However, considering that approximately half the world wide population of preschool children are afflicted by anemia, with a prevalence as high as 64.6% in Africa and 47.7% in Asia, and that we know anemia prevalence is higher in the group of children less than two years old, we could possibly assume that anemia in this specific age group is an essential global public health problem, mainly in low income countries(Mamabolo and Alberts, 2014, Ritu et al., 2017). According to previous studies in Iran, the prevalence of anemia between children is 15% in this area (Sayyari et al., 2006).

Blood Hb levels were evaluated in patients to determine anemia prevalence between children with growth retardation. Ferritin levels among patients were measured to evaluate prevalence of iron deficiency between children with growth failure. Mid cell volume (MCV) of the patients was measured to evaluate the prevalence rate of microcytic anemia in growth retarded children and adolescents. Table 1 shows data about these variables.

Also these variables were investigated according to cut off points which is present in table 2.

Anemia prevalence was defined between 4 different age groups and the results were shown in table 3.

According to Gomez et al., (1955) and Waterlow (1972) classification of undernutrition in children is rearranged in table 4, the patients were divided in 3 categories (mild, intermediate and severe malnutrition). There was significant relation between serum ferritin levels and undernutrition. Serum levels of ferritin decreases with increasing undernutrition.

Table 1. Blood Hb, ferritin and MCV maximum and minimum rates

Variable	Maximum rate	Minimum rate	Average rate	Standard deviation
Blood Hb	13.9gr/dl	9.2 gr/dl	11.8 gr/dl	1.25
Ferritin	180 µg/L	5 µg/L	43 µg/L	36.6
MCV	83fm	69fm	70fm	

Table 2. Variables according to cut off point

Variable	Cut off point	Number of patients with lower cut off point	percentage	P value(between two sexes)
Hb	<11.3 g/dL	37	37%	0.028
ferritin	<15 µg/L	25	25%	0.06
MCV	<80	29	29%	0.08

Anemia prevalence according to age associated normal Hb was 25% in boys and 41% in girls which was significantly different.

In this study we resulted that the prevalence of anemia in growth retarded children was 35.9%. In a similar study, Anderson et al (Monchy et al., 2008) showed that the prevalence of anemia in children with growth failure in New Zealand was 71%. They also resulted that microcytic anemia prevalence between the patients was 20 % which matches with our results with 21% of prevalence. Anderson et al also resulted that 6% of children with growth retardation had lower ferritin levels than normal value. In the present study the prevalence of low ferritin levels was 1.6%. Socio-economic status, indicated by place of life was an essential factor determining the risk of anemia.

In our survey, anemia was detected in 41% of the girls compared to 25% of the boys. Similarly, Kara et al and Yavuz et al also noticed a higher prevalence of anemia in girls compared with boys, (Kara et al., 2006, Yavuz et al., 2004). The lower incidence of anemia among pubertal boys might possibly be explained by the physiological surge in hemoglobin level triggered by sexual maturation and consequently by lowering the requirements after passing of the growth spurt. In girls, the phenomenon of menarche and menstrual irregularities reduce an expected age related increasing amount of hemoglobin concentration (Kara et al., 2006). The anemia found in this study was microcytic, hypochromic anemia, as showed by a low MCV. Anemia of this type has been known to be connected with iron deficiency(Torabizadeh et al., 2004).

The prevalence of anemia and iron deficiency in this study is much less than previous reports about normal kids from this age group in other regions of Iran as well as the reports of other developing countries (Akramipour et al., 2008, Hashizume et al., 2003), that indicates the important role of iron deficiency in growth failure of

Table 3. Anemia prevalence in 4 different age groups

Age group	Number of Patients	Percentage of patients showed anemia based on normal Hb	Average µg/dL	Patients with ferritin lower than normal value
6months- 2 years old	11	42.8%	38	-
2-6 years old	31	35%	29.5	-
6-12years old	39	20%	52.44	-
12-18years old	19	8.3%	49.2	2(10.5%)

Table 4. Gomez and Waterlow malnutrition classification in children

Grade of malnutrition		Weight for age	Height for weight	Weight for height
0	Normal	›90	›95	›90
1	Mild	75-90	90-95	81-90
2	Moderate	60-74	85-89	70-80
3	Severe	< 60	<85	<70

children. In addition, we cannot find any considerable correlation between hemoglobin level and serum ferritin revealing that iron status was not likely an essential determinant agent of anemia in the evaluated population. These results are actually in opposition to Hashismue et als study (Gomez et al., 1955). Brined et als study in Bangladesh (Briend et al., 1990) evaluated growth rate of 694 children from rural regions. They resulted that children drinking water with iron values more than 1mg/lit, were significantly higher than children drinking water containing less than 1mg/lit. They concluded that iron deficiency causes growth retardation of children in poor societies which in conformity with our study.

CONCLUSION

Since iron deficiency leads to growth failure in children through different ways such as decreasing oxygen transport capacity, decreasing energy production and decreasing appetite, blood iron levels monitoring in children is of great importance. The results of this study suggest that treatment of growth retarded children with iron supplements can improve their growth. Because data about prevalence of IDA in Iranian children is limited, we suggest further studies to be performed to define an average range of iron concentrations in accordance with other microelements such as zinc and copper in children in this area.

AUTHORS' CONTRIBUTION

Whole authors were in the same.

FINANCIAL DISCLOSURE

There is no conflict of interest.

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