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CDKN2A/2B rs10811661 gene polymorphism and sedentary life style factors, their risk association with type 2 diabetes mellitus in Indian population–A case control study

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ABSTRACT

CDKN2A/2B is a major gene in pancreatic β-cell dysfunction and regeneration related with Type-2 diabetes, Obesity, Insulin resistance and CVD. Current study analyses the consortium of *CDKN2A/2B* polymorphism in 369 Type 2 Diabetes cases and 100 controls in Indian population. The study was done by PCR-RFLP technique to study the *CDKN2A/2B* polymorphism in the study subjects. Study contemplated that CDKN2A/2B C/T genotype distribution between cases and controls were found to be significant (0.0001). Higher CDKN2A/2B T allele frequency (0.31%) in cases as compare to control (0.13%). CDKN2A/2B CT and TT had 3.16 (1.84-5.42), 5.84 (1.75-19.45) Odd ratios with 95% class intervals. HbA1C, Cholesterol, HDL and LDL were responsible for disease, and significant results were observed in biochemical parameters. On comparing biochemical parameters with sedentary life style factors, it was observed that PPG and FPI, HDL exhibited positive relation with smoking at p(0.04), (0.02) and (0.07) respectively, alcoholism with HDL at p(0.05), Non vegetarian food with Triglycerides at p(0.005),and Exercise (not doing) with PPG, HbA1C, FPI, HDL, LDL and triglycerides at p (0.02, 0.0001, 0.04, 0.0001 and 0.0003) respectively. Remaining parameters manifest no significance. The epilogue of the study contemplates affiliation of *CDKN2A/2B rs10811661* gene polymorphism with possibility of Type 2 Diabetes Mellites. HbA1C, Cholesterol, HDL and LDL increases the risk of occurrence of T2DM in subject population. Significant association between biochemical parameters with sedentary life style factor increases the danger of Type 2 Diabetes Mellites in Indian population.

KEY WORDS: TYPE 2 DIABETES MELLITES; CDKN2A/2B GENE; ALLELE FREQUENCY; GENETIC ALTERATION; POLYMORPHISM

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INTRODUCTION

In the recent past the Prevalence of Diabetes mellitus has been increasing as major widespread chronic non communicable diseases worldwide, and with urbanization altered lifestyle characterised with reduced physical activity and obesity, the pace of occurrence has escalated. ("WHO | Diabetes programme," n.d.). The International Diabetes Federation (IDF) has confirmed that the pervasiveness of diabetes will growing from 451 million in 2017 to 693 million by 2045, (Cho et al., 2018). The pervasiveness of T2DM continue to increase worldwide with growing sedentary lifestyle of people and accounts for more than 80% of diagnosed cases currently (Murea et al., 2012). While incidents of T2DM varies with age, sex and population. It is estimated that worldwide age-standardized adult diabetes is >9.2%, and confirmed that >347 million adults have diabetes, (Danaei et al., 2011). T2DM can be diagnosed by FPG, PPG, HbA1C and many clinicopathological parameters which are associated with disease. Sedentary life style induced Physical inactivity and obesity also contribute to prevalence of T2DM (Committee, 2009, Mehramiz et al., 2018). Decrease insulin supply attributable to insulin resistance causes T2D mostly induced because of genetic and environmental factors in early phases, whereas Decline in β-cell function that cause tissues to become resistant to insulin is gradual event in the later phase, (Schäfer et al., 2011). Researchers have estimated that by 2030, ~552 million individuals globally will have diabetes, with Asians becoming the most affected group, (Chan et al., 2009; Whiting et al., 2011). Genetic linkage analyses and GWAS have confirmed variants in genes that contribute toward the development of T2DM, (Bao et al., 2012, Mehramiz et al., 2018 and Cho et al., 2018).

CDKN2A/2B rs10811661 Cycline-dependent kinase inhibitor-2A/B has been reported as one of the prospect gene responsible for type 2 diabetes based on its chromosomal position (9) and its vital task in beta cell function and revival. (Voight et al., 2010) *CDKN2A/2B* in T2D impairs immune system functioning which altered the cytokine creation and leukocyte turn on in tissues such as liver, adipose tissue, pancreas, and the vascular wall. Immune cells also regulate obesity induced glucose intolerance and insulin resistance in T2D, (Hannou et al., 2015). An interrelation between SNPs adjacent to *CDKN2A/B* gene and impaired first phase glucose-induced insulin production have been confirmed instead of deficiency in glucose tolerance or insulin sensitivity, (Hart et al., 2010; Hribal et al., 2011) indicating the connection of *CDKN2A/B* in pancreatic β-cell. Candidate gene approaches have also examined variants in this locus related with various types of cancer like breast cancer, acute lymphoblastic leukaemia, ovarian cancer, glioma, malignant melanoma, pancreatic cancer, glaucoma and nasopharyngeal neoplasm, presenting that the chromosomal area harbours genes involved in a few different physiological processes, (Foulkes et al., 1997). A polymorphism on chromosome 9p (rs10811661), located 125 kb upstream of the CDKN2B and CDKN2A genes has been related with type 2 diabetes in the genome-wide association (GWA) studies, (Scott et al., 2007; Voight et al., 2010; Zeggini et al., 2007 Mehramiz et al., 2018).

In the Danish inhabitants research study, comprising 5,970 adult individuals, it was confirmed that *CDKN2A/2B* C allele showed increased level of insulin generation in reciprocation to an oral glucose load compared with individuals having the TT genotype. (Grarup et al., 2007) similar outcomes were also obtained in a research work of 5,327 non-diabetic Finnish inhabitants, (Scott et al., 2007). These genotypic relation results was replicated in several ethnic groups including Danish, Norwegian, French, Korean, Japanese and Chinese participants. (Duesing et al., 2008; Grarup et al., 2007; Hertel et al., 2008; Lee et al., 2008; Wu et al., 2008) but not confirmed in African-Americans and Pima Indians, (Lewis et al., 2008; Rong et al., 2009). Thus, to acquire supplementary outcome on the consortium of the rs10811661 polymorphism with impaired glucose tolerance and pathophysiological quantitative traits, i.e. measures of beta cell function and insulin sensitivity, biochemical parameters we have conducted this particular study on well-characterised samples of Indian population.

MATERIAL AND METHODS

The present study on "patients-healthy persons" was completed at the Jamia Millia Islamia, New Delhi, in Medical Biotechnology Laboratory, New Delhi, where total of 469 individuals were taken. Out of 469 there were 369 newly diagnosed T2DM cases (patients) and 100 non-T2DM controls (Healthy persons). A proper inclusion/exclusion criterion for T2DM cases and healthy controls population was followed. The samples were collected after the ethical clearance from various institutional ethical committees, like Jamia Millia Islamia, New Delhi, Gurukula Kangri University, Haridwar and from all sample collection sites. Patient included in the present study were screened and sample collection was done after informed consent of the concerned individuals (diabetic/non-diabetic). Patient's information was collected in standardized pretexted questionnaires and it was entered later on in database. Patients Performa for recording clinical data of each patient and patient consent was maintained throughout the course of study. Written informed assent was obtained from

every individual involved in the research work. All the clinical factors such as fasting plasma glucose, post-prandial plasma glucose, cholesterol, triglycerides, were taken care off as per approved criteria. Total 2 ml of Blood samples were taken in EDTA coated vacutainer of Type- 2 diabetic patients as well of healthy persons. The non-diabetic (healthy persons) were taken as control for the present study. (The samples were collected in the hospital by the expert medical professional according to guidelines provided by ICMR/GCP.)

DNA extraction was done using phenol chloroform method from collected blood samples. Genomic DNA was examined on 2% agarose gel to confirm and observed under UV transilluminator. DNA quantity came out to be 45-50 ng. A260/A280 ratio came around 1.8 and perfect DNA band with no smearing was resulted after agrose gel electrophoresis. Which state that the extracted DNA was of pure, without impurity and intact. Extracted DNA was then amplified to determine the genotypes of *CDKN2A/2B* by a- specific primers forward: 5'-CCGGC-CCATTTTCTTTGTCA-3' and reverse: 5'-CAAAGCGCTGG-GATCATAGG-3' using thermos cycler. PCR was imple-mented in 20µl reaction volume having1 µl DNA, 1 µl primer each forward and reverse, 10 µl Taq polymerase and 7 µl nuclease free water (ddH_2o). The PCR was con-ducted with beginning denaturation at 94°C for 3 min-utes, come next with 35 cycles of denaturation's at 94°C for 30 seconds, annealing at 61°C for 30 seconds, initial extension at 72°C for 40 seconds and final extension at 72°C for 5 minutes. The PCR product of 232 bp was seen under UV trans illuminator. RFLP (Restriction Fragment Length Polymorphism) *CDKN2A/2B* polymorphism was done by digesting 6 µl PCR product (amplified genes) with restriction enzyme BspHI 2.5 units in 10 µl reaction mixture minimum 30 minutes or maximum overnight at 37° C. The restriction enzyme recognizes the sequence. Digested DNA was examined by 3% Agarose Gel Elec-trophoresis. DNA band showed 232bp C allele: uncut T allele: 164+68 bp.

Statistical analysis: Genotype frequencies between cases and controls were calculated using Chi-square test. The values <5 were analysed by Fisher exact test. The Fisher Exact test is a test of significance conducted for more accuracy of results in 2 by 2 tables, especially when the same size is small. Allele frequency was calculated by Hardy-Weinberg Equilibrium equation. The relation between *CDKN2A/2B* genotype and risk of T2DM were drawn by calculating the odd-ratios (OR) with 95% con-fidence intervals. In the parametric and nonparametric data, p value <0.05 was considered statistically signifi-cant. Analysis of Variance -One-Way ANOVA was used to compare the clinicopathological parameters geno-type of T2DM cases and control where p value <0.05 was considered significant. Comparison of biochemical parameters with sedentary life style parameters by one-way Anova test, p value <0.05 considered significant. Statistical analyses were carried out using SPSS soft-ware, version 19.0 (IBM Corp., Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Biochemical characteristic of research subjects analysed in the present study has been summarised in Table 1: Among these characteristics Hip, weight, BMI, FPG, PPG, HbA1C, BP (s, d), Cholesterol, LDL and triglycerides are significantly associated with risk of T2DM as compare of means ± sd between cases and control (Student'-t test applied).

Table 1. Comparison of biochemical parameters among T2D cases and controls.

Variables	Cases (Mean+SD)	Controls (Mean+SD)	P Value
Hip	35.44+3.22	34.87+1.88	0.09
Waist	32.0+3.77	31.65+2.50	0.37
Weight	78.26+14.82	70.74+7.79	<0.0001
BMI	28.45+5.24	24.83+2.33	<0.0001
FPG	137.3+33.63	90.22+7.10	<0.0001
PPG	211.6+70.05	135+13.02	<0.0001
HbA1C	7.14+1.10	5.74+0.53	<0.0001
FPI	10.18+8.36	8.65+0.71	0.07
BP (systolic)	144.4+18.46	106.1+10.39	<0.0001
BP (diastolic)	104.5+44.41	75.85+10.91	<0.0001
Cholesterol	245.5+15.15	152.6+18.82	<0.0001
HDL	46.68+11.35	46.21+8.69	0.69
LDL	192.3+29.12	106.4+19.92	<0.0001
Triglycerides	357.9+99.10	141.0+5.52	<0.0001

*note: data presented as means ± sd; p-value<0.05 considered significant.

The difference seen in the genotype among patients and healthy person was found significant (p<0.0001) (Table 2). It was seen that high percentage of heterozy-gous CT 148 (40.10%) and mutant TT 41 (11.11%) genotype was found in patients compared to control heterozygous CT 20 (20%) and TT 03 (3%) while lower CC 180 (48.78%) geno-type in patients compared to control homozygous CC 77 (77%) genotype. The higher allele frequency of T allele (0.31) was observed in T2DM patients compared to con-trol (0.13).

Table 2. Genotypic distribution and allele frequencies of *CDKN2A/2B* gene among T2DM patients and Healthy controls.

CDKN2A/2B	CC (%)	TT n(%)	CT n(%)	p value	Allele frequency	
					C allele	T allele
Patients (369)	180	41	148	<0.0001	0.69	0.31
Controls (100)	77	03	20		0.87	0.13

*note: data presented as n (%); p-value<0.05 considered significant. genotype frequency by chi-square test & allelic frequency calculated by hardy weinberg equation.

Odd ratio with 95% confidence intervals was drawn for each group to estimate the degree of association between CDKN2A/2B genotype and risk of T2DM in Indian patients presented in Table 3. Compared to the CC genotype, the OR 3.16 (1.84-5.42) for heterozygous and OR 5.84 (1.75-19.45) for mutant homozygous were estimated suggesting a possible dominant effect of CDKN2A/2B polymorphism on T2DM risk.

Table 3. Risk of T2DM associated with CDKN2A/2B genotype.

CDKN2A/2B Genotype	T2D patients	Healthy controls	OR (95% CI)
CC	180	77	(ref)
TT	41	03	5.84 (1.75-19.45)
CT	148	20	3.16 (1.84-5.42)

*note: data presented as n (%) association estimates by computing odd ratio (or) with 95% confidence intervals.

The risk associated with various biochemical parameters like Waist- Hip ratio, weight, BMI, FPG, PPG, HbA1C, BP (s, d), Cholesterol, LDL and triglycerides have been calculated and presented in (Table 4). Analysis results of the relation linking CDKN2A/2B rs10811661 C/T polymorphism genotypes and the biochemical parameters have also been illustrated. The *CDKN2A/2B rs10811661* "TT" genotype exerts significant effect on HbA1C, Cholesterol, HDL-C and LDL-C level in both patients and controls (*P* values = 0.02, 0.04, 0.02 and 0.03 respectively). Individuals harbouring this genotype seem to have a significantly higher HbA1C, Cholesterol, HDL-C and LDL-C as compared to carriers of the CC genotype. No significant effect of the *CDKN2A/2B* rs10811661 genotypes on the FPG, FPI, PPG and Triglycerides was observed.

A Comparison of biochemical parameters with sedentary life style factors was conducted to assess the dominance of these factors in biochemical variables, by one-way Anova Test where, p value of <0.05 was considered significant. In this regard all the Lifestyle factors like smoking, consumption of Alcohol, physical activeness was assessed individually with all the biochemical parameters to identify the dominant association of these factors along with Biochemical parameters. On comparing the habit of smoking with biochemical variables it was observed that PPG and FPI, HDL showed significant association with smoking were p value of 0.04, 0.02 and 0.07 respectively was recorded as mentioned in (table 5). It was observed that the patients who smoke have higher PPG, FPI and HDL as compared to them who don't smoke. According to the observation made these 3 parameters

Table 4. The relation between *CDKN2A/2B* genotypes and the investigated clinical parameters.

Variables	CDKN2A/2B genotypes			P Value
	CC (Mean+SD)	TT (Mean+SD)	CT (Mean+SD)	
FPG	137.6+37.37	145.3+27.23	134.7+30.07	0.19
PPG	210.7+52.84	219.0+42.65	210.5+91.50	0.77
HbA1C	7.16+1.11	7.55+1.24	7.01+1.04	0.02
FPI	9.63+1.38	9.15+1.30	10.33+10.0	0.56
Cholesterol	247.5+14.21	243.9+15.36	243.5+15.97	0.04
HDL	47.82+12.51	48.80+8.91	44.72+10.18	0.02
LDL	192.1+29.75	182.1+35.01	195.3+25.96	0.03
Triglycerides	358.6+97.18	331.9+82.41	364.3+104.9	0.17

*note- note: data presented as means ± sd; p-value<0.05 considered significant. analysis of variance (anova) - one-way anova from summary data. abbreviations- fpg- fasting plasma glucose, ppg- post prandial plasma glucose, hba1c- haemoglobin a1c test, hdl- high density lipoprotein, ldl- low density lipoprotein.

Table 5. Comparison of biochemical parameters with sedentary life style factor (Smoking) among T2Dcases and controls.

Variables	Smoking			P value
	Current (Mean+SD)	Former (Mean+SD)	Never (Mean+SD)	
FPG	139.4+37.95	135.1+48.5	135.9+27.95	0.60
PPG	221.8+95.85	218.3+65.08	203.0+39.57	0.04
HbA1C	7.22+1.16	6.87+1.12	7.11+1.05	0.35
FPI	10.58+9.86	9.18+1.17	9.96+7.49	0.02
Cholesterol	246.7+14.92	244.4+21.81	244.7+14.55	0.45
HDL	47.66+10.95	41.70+12.72	46.43+11.43	0.07
LDL	191.7+29.97	194.2+35.55	192.5+27.88	0.92
Triglycerides	351.8+93.41	359.7+111	362.5+102.3	0.66

* note: data presented as means±sd; p-value<0.05 considered significant. analysis of variance (anova) - one-way anova from summary data.

increases with nicotine consumption which comes with smoking. However, rest of the biochemical parameters showed no significant association with smoking as all of them were above the significant p value of 0.05.

On comparing (Alcoholism) as one of the factors of lifestyle with all the 8 Biochemical parameters taken amongst cases and control samples, only 1 parameter showed significant association which was HDL Cholesterol (see table 6). The consumption of Alcohol reflected significant association with HDL cholesterol, were p value of 0.05 was found. The patients with increase alcohol consumption have increased HDL-C level. Rest of the biochemical parameters showed no significant association with sedentary life style factors the p value was more than 0.05.

Similarly, on comparing Non-Vegetarian food (as a factor of Sedentary lifestyle) habits among T2Dcases and controls with Biochemical parameters it was observed that Triglycerides with significant p value of 0.05 can be associated with consumption of Non- Vegetarian food (as shown in table -7). It means that patients who consume Non-vegetarian food as major part of their Diet have increased Triglycerides level. Rest of the biochemical parameters showed no significant association with sedentary life style factors.

Similarly, when Exercise factor of the Sedentary lifestyle was taken among T2Dcases/ controls and were compared with given 8 Biochemical parameters, 5 amongst them highlighted positive significant relation because the p value of all these 5 variables were observed to be < then 0.05) as presented in table -8). It was observed that exercise is associated with PPG, HbA1C, FPI, HDL, LDL and triglycerides, with significant p value of 0.02, 0.0001, 0.04, 0.0001 and 0.0003 respectively. The patients with less physical activity or less exercise habit have increased PPG, HbA1C, FPI, HDL, LDL and triglycerides level. Rest of the biochemical parameters showed no significant association with sedentary life style factor parameters since the p vale of remaining variables were recorded more than p value of 0.05.

GWAS have provided information's on a broad scale, to see the relation of many gene variants with complex

Table 6. Comparison of biochemical parameters with sedentary life style factor (Alcoholism) among T2D cases and controls.

Variables	Alcoholism			P value
	Current (Mean+SD)	Former (Mean+SD)	Never (Mean+SD)	
FPG	135.6+29.22	142.8+20.73	138.2+37.69	0.61
PPG	215+87.59	214.+27.47	208.5+55.07	0.67
HbA1C	7.16+1.11	7.62+1.26	7.09+1.07	0.61
FPI	10.98+12.67	9.75+1.50	9.56+1.33	0.74
Cholesterol	246.0+14.0	245.2+15.80	245.1+16.04	0.85
HDL	46.87+10.74	51.76+10.48	46.08+11.84	0.05
LDL	193.6+27.19	179.5+35.38	192.3+29.94	0.16
Triglycerides	364.5+89.81	347.1+104.2	353.6+99.10	0.52

* note: data presented as means±sd; p-value<0.05 considered significant. analysis of variance (anova) - one-way anova from summary data.

Table 7. Comparison of biochemical parameters with sedentary life style factor (Non-Vegetarian food habit) among T2Dcases and controls.

Variables	Non-Vegetarian food habit			P value
	High (Mean+SD)	No (Mean+SD)	Normal (Mean+SD)	
FPG	137.1+35.04	132.3+20.07	140.2+38.28	0.18
PPG	218.7+111.0	208.0+26.04	209.3+53.51	0.47
HbA1C	7.09+1.19	7.19+1.09	7.15+1.06	0.79
FPI	9.52+1.39	9.61+1.35	9.60+1.44	0.86
Cholesterol	244.5+15.74	245.9+15.10	245.9+14.89	0.73
HDL	46.05+10.13	47.26+12.76	46.74+11.26	0.75
LDL	193.0+28.29	190.5+31.55	192.8+28.34	0.79
Triglycerides	334.9+90.79	381.5+105.1	358.6+97.82	0.005

*note: data presented as means±sd; p-value<0.05 considered significant. analysis of variance (anova) - one-way anova from summary data.

diseases like T2D and CVD.(Hannou et al., 2015) In the current research work significant difference in distribution of CDKN2A/2B genotype among T2DM cases and controls was observed. An independent association of mutant TT and heterozygous genotype CT were found to be related with increased risk of T2DM. It was found that TT and CT genotype in patients showed 05 to 2-fold increase as compare to healthy control. The CDKN2A/2B rs10811661 "TT" genotype exerts significant effect on HbA1C, Cholesterol, HDL-C and LDL-C level in both patients and controls (*P* values = 0.02, 0.04, 0.02 and 0.03 respectively). HbA_{1c} is now approved in developed countries as a diagnostic as well as monitoring test for (type 2) diabetes although the debate regarding its applicability for diagnosis still prevails (d'Emden et al., 2012; "Diagnosis and Classification of Diabetes Mellitus," 2010; "WHO | Diabetes programme," n.d.; Long et al., 2017).

HbA1c has been used as a specific indication of glycaemic control in all type of diabetes. In T2DM patients, HbA1c emergence is a direct function of the average blood glucose concentration. Utilization of HbA1c as a diagnostic test has edge, international standardization (Committee, 2009). It provides an index of glycaemia over the entire 120-day lifespan of the red blood cell, (Tahara and Shima, 1995). In our study CDKN2A/2B genotype showed significant association with increasing level of HbA1C. In CDKN2A/2B genotype CT and TT showed 7.01±1.04 and 7.01±1.04 with p value of 0.02 showing their association with T2DM because the significant p value is less than 0.05

Table 8. Comparison of biochemical parameters with sedentary life style factor (Exercise) among T2Dcases and controls.

Variables	Exercise		P value
	Yes (Mean+SD)	No (Mean+SD)	
FPG	139.3+37.50	135.4+29.46	0.27
PPG	220.1+91.67	203.5+38.34	0.02
HbA1C	6.87+0.93	7.43+1.20	<0.0001
FPI	10.60+9.06	9.23+1.24	0.04
Cholesterol	244.3+16.01	246.7+14.24	0.13
HDL	48.17+11.36	45.27+11.19	0.01
LDL	184.5+34.48	199.7+20.36	<0.0001
Triglycerides	339.2+96.97	375.8+98.04	0.0003

*note: data presented as means±sd; p-value<0.05 considered significant. analysis of variance (anova) -one-way anova from summary data.

Cholesterol is one of the major biochemical parameters for risk of T2DM and cardiovascular diseases. Individual with diabetes commonly have same level of total cholesterol levels and the 'good' (HDL) cholesterol as the general population. However, on an average the level of 'bad (LDL) cholesterol and triglycerides is more in individual with diabetes as compare to individual without diabetes.This is primarily because diabetes can upset the equilibrium between 'good' (HDL) and 'bad' (LDL) cholesterol levels in several ways("Cholesterol," n.d.). In case of CDKN2A/2B genotype the cholesterol showed significant association with p value of 0.004 which was less than the significant p value of 0.05. Although plasma LDL cholesterol level is usually normal in type 2 diabetic patients, metabolism of LDL is significantly modified.(Vergès, 2005). In case of T2DM attribute important modification of both LDL and HDL which are likely to play an major role in the occurrence of atherosclerosis (Vergès, 2009). Type 2 diabetes is related with reduction of the HDL (Visser et al., 2017). In our study the LDL and HDL show significant association with CDKN2A/2B CT and TT genotype. Genotype CT have LDL 195.3±25.96, and TT have LDL value of 182.1±35.01 and significant p value of 0.03. In HDL CDKN2A/2B CT, TT genotypes have 44.72±10.18, 48.80±8.91 and p value is 0.02 which show significant association.

Other biochemical parameters also showed significant association with type 2 diabetes a comparative analysis between T2DM cases and control in the study showed that weight, BMI, FPG, PPG and blood pressure p value (0.0001). In our study we compare biochemical parameters with sedentary life style factors such as Smoking, Alcoholism, Non vegetarian food habits and physical Exercise, it was observed that PPG and FPI, HDL showed significant association with smoking with p values of 0.04, 0.02 and 0.07 respectively, Similarly alcoholism is associated with HDL cholesterol with significant p value of 0.05 found, Non-Vegetarian food habit is associated with Triglycerides, showing significant p value of 0.005 and exercise is associated with PPG, HbA1C, FPI, HDL, LDL and triglycerides with significant p values of 0.02, 0.0001, 0.04, 0.0001 and 0.0003 respectively. The patients with less physical activity habit have increased PPG, HbA1C, FPI, HDL, LDL and triglycerides level. Rest of the biochemical parameters showed no significant association with sedentary life style factor parameters because all the remaining parameters had p value more than the significant value of 0.05. Researchers confirmed that the *CDKN2A*-rs10811661 polymorphism was found to be major constituent related with prediabetes in the model adjusted for age, sex, obesity, blood pressure, dyslipidaemia, socio-economic status, and lifestyle factors, (Binh et al., 2015). Obesity, which is one of main factor responsible for insulin resistance and dysfunction of beta cell resulted in development of prediabetes, (Kahn and Flier, 2000).

Studies suggested that CDKN2A/2B genotype is also related with CVD and these risks related with unhealthy dietary pattern. High BMI showed that obese with a TT genotype had a higher level of TG, TG/HDL ratio, compared to individuals with a normal BMI. Moreover, the presence of a TT genotype was associated with increased risk of hypercholesterolemia, insulin resistance and CVD. These effects were more pronounced in the subgroup with low physical activity and a high dietary energy intake, (Mehramiz et al., 2018) .The study came to an end that CDKN2A/2B rs10811661 gene polymorphism was found to be related with risk of T2DM and risk was connected with heterozygosity and mutant homozygosity. HbA1C, Cholesterol, HDL-C and LDL-C are possible risk factor for developing T2DM and significantly altered biochemical parameter in T2DM patients. Comparison of biochemical parameters with sedentary life style factors such as Smoking, Alcoholism, non-vegetarian food habits and physical Exercise, was conducted and it was observed that some biochemical parameters showed significant association. These compelling results of the study require further validation on larger population. Carrying out a similar study on T2DM female patients to reveal combined gender/polymorphism effect. The Study signifying the association between sedentary life style and T2M also urges patients to maintain/control BMI, HbA1c, LDL-C, triglyceride levels, body weight and change in life style, food habits, reduce the consumption of alcohol and cigarette smoking meanwhile maintaining a proper healthy life style with routine exercise regime and healthy eating habits. in order to avoid the complications associated with T2DM.

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REFERENCES

Bao, X.Y., Xie, C., Yang, M.S., 2012. Association between type 2 diabetes and CDKN2A/B: a meta-analysis study. Mol. Biol. Rep. 39, 1609–1616. https://doi.org/10.1007/s11033-011-0900-5

Binh, T.Q., Thu, N.T.T., Phuong, P.T., Nhung, B.T., Nhung, T.T.H., 2015. CDKN2A-rs10811661 polymorphism, waist-hip ratio, systolic blood pressure, and dyslipidemia are the independent risk factors for prediabetes in a Vietnamese population. BMC Genet. 16, 107. https://doi.org/10.1186/s12863-015-0266-0

Chan, J.C., Malik, V.S., Jia, W., Kadowaki, T., Yajnik, C.S., Yoon, K.-H., Hu, F.B., 2009. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA 301, 2129–2140. https://doi.org/10.1001/jama.2009.726

Cho, N.H., Shaw, J.E., Karuranga, S., Huang, Y., da Rocha Fernandes, J.D., Ohlrogge, A.W., Malanda, B., 2018. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res. Clin. Pract. 138, 271–281. https://doi.org/10.1016/j.diabres.2018.02.023

Cholesterol [WWW Document], n.d. URL https://www.diabetesaustralia.com.au/cholesterol (accessed 4.25.19).

Committee T.I.E., 2009. International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes. Diabetes Care 32, 1327–1334. https://doi.org/10.2337/dc09-9033

d'Emden, M.C., Shaw, J.E., Colman, P.G., Colagiuri, S., Twigg, S.M., Jones, G.R.D., Goodall, I., Schneider, H.G., Cheung, N.W., Australian Diabetes Society, Royal College of Pathologists of Australasia, Australasian Association of Clinical Biochemists, 2012. The role of HbA1c in the diagnosis of diabetes mellitus in Australia. Med. J. Aust. 197, 220–221.

Danaei, G., Finucane, M.M., Lu, Y., Singh,2011 Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Blood Glucose), 2011. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. Lancet Lond. Engl. 378, 31–40. https://doi.org/10.1016/S0140-6736(11)60679-X

Diagnosis and Classification of Diabetes Mellitus, 2010. Diabetes Care 33, S62–S69. https://doi.org/10.2337/dc10-S062

Duesing, K., Fatemifar, G., Charpentier, G., Marre, M., 2008. Strong association of common variants in the CDKN2A/CDKN2B region with type 2 diabetes in French Europids. Diabetologia 51, 821–826. https://doi.org/10.1007/s00125-008-0973-4

Foulkes, W.D., Flanders, T.Y., Pollock, P.M., Hayward, N.K., 1997. The CDKN2A (p16) gene and human cancer. Mol. Med. 3, 5–20.

Grarup, N., Rose, C.S., Andersson, E.A., Andersen, G., , 2007. Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. Diabetes 56, 3105–3111. https://doi.org/10.2337/db07-0856

Hannou, S.A., Wouters, K., Paumelle, R., Staels, B., 2015. Functional genomics of the CDKN2A/B locus in cardiovascular and metabolic disease: what have we learned from GWASs? Trends Endocrinol. Metab. 26, 176–184. https://doi.org/10.1016/j.tem.2015.01.008

Hart, L.M. 't, Simonis-Bik, A.M., Nijpels, G., Haeften, T.W. van, Schäfer, 2010. Combined Risk Allele Score of Eight Type 2 Diabetes Genes Is Associated With Reduced First-Phase Glucose-Stimulated Insulin Secretion During Hyperglycemic Clamps. Diabetes 59, 287–292. https://doi.org/10.2337/db09-0736

Hertel, J.K., Johansson, S., Raeder, H., Midthjell, K., Lyssenko, V., Groop, L., Molven, A., Njølstad, P.R., 2008. Genetic analysis of recently identified type 2 diabetes loci in 1,638 unselected patients with type 2 diabetes and 1,858 control participants from a Norwegian population-based cohort (the HUNT study). Diabetologia 51, 971–977. https://doi.org/10.1007/s00125-008-0982-3

Hribal, M.L., Presta, I., Procopio, T., 2011. Glucose tolerance, insulin sensitivity and insulin release in European non-diabetic carriers of a polymorphism upstream of CDKN2A and CDKN2B. Diabetologia 54, 795–802. https://doi.org/10.1007/s00125-010-2038-8

Kahn, B.B., Flier, J.S., 2000. Obesity and insulin resistance. J. Clin. Invest. 106, 473–481. https://doi.org/10.1172/JCI10842

Lee, Y.-H., Kang, E.S., Kim, S.H., , 2008. Association between polymorphisms in SLC30A8, HHEX, CDKN2A/B, IGF2BP2, FTO, WFS1, CDKAL1, KCNQ1 and type 2 diabetes in the Korean population. J. Hum. Genet. 53, 991–998. https://doi.org/10.1007/s10038-008-0341-8

Lewis, J.P., Palmer, N.D., Hicks, P.J., Sale, M.M., Langefeld, C.D., Freedman, B.I., Divers, J., Bowden, D.W., 2008. Association Analysis in African Americans of European-Derived Type 2 Diabetes Single Nucleotide Polymorphisms From Whole-Genome Association Studies. Diabetes 57, 2220–2225. https://doi.org/10.2337/db07-1319

Long, M., Wang, C., Liu, D., 2017. Glycated hemoglobin A1C and vitamin D and their association with diabetic retinopathy severity. Nutr. Diabetes 7, e281. https://doi.org/10.1038/nutd.2017.30

Mehramiz, M., Ghasemi, F., Esmaily, H., 2018. Interaction between a variant of CDKN2A/B-gene with lifestyle factors in determining dyslipidemia and estimated cardiovascular risk: A step toward personalized nutrition. Clin. Nutr. 37, 254–261. https://doi.org/10.1016/j.clnu.2016.12.018

Murea, M., Ma, L., Freedman, B.I., 2012. Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. Rev. Diabet. Stud. RDS 9, 6–22. https://doi.org/10.1900/RDS.2012.9.6

Rong, R., Hanson, R.L., Ortiz, D., Wiedrich, C., Kobes, S., Knowler, W.C., Bogardus, C., Baier, L.J., 2009. Association analysis of variation in/near FTO, CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761, and CDKN2B with type 2 diabetes and related quantitative traits in Pima Indians. Diabetes 58, 478–488. https://doi.org/10.2337/db08-0877

Schäfer, S.A., Machicao, F., Fritsche, A., Häring, H.-U., Kantartzis, K., 2011. New type 2 diabetes risk genes provide new insights in insulin secretion mechanisms. Diabetes Res. Clin. Pract. 93 Suppl 1, S9-24. https://doi.org/10.1016/S0168-8227(11)70008-0

Scott, L.J., Mohlke, K.L., Bonnycastle, L.L., Willer, C.J., Li, 2007. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316, 1341–1345. https://doi.org/10.1126/science.1142382

Tahara, Y., Shima, K., 1995. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care 18, 440–447.

Vergès, B., 2009. Lipid modification in type 2 diabetes: the role of LDL and HDL. Fundam. Clin. Pharmacol. 23, 681–685. https://doi.org/10.1111/j.1472-8206.2009.00739.x

Vergès, B., 2005. New insight into the pathophysiology of lipid abnormalities in type 2 diabetes. Diabetes Metab. 31, 429–439.

Visser, B.J., de Vries, S.G., Vingerling, R., Gritter, M., Kroon, D., 2017. Serum Lipids and Lipoproteins during Uncomplicated Malaria: A Cohort Study in Lambaréné, Gabon. Am. J. Trop. Med. Hyg. 96, 1205–1214. https://doi.org/10.4269/ajtmh.16-0721

Voight, B.F., Scott, L.J., Steinthorsdottir, V., Morris, GIANT Consortium, 2010. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat. Genet. 42, 579–589. https://doi.org/10.1038/ng.609

Whiting, D.R., Guariguata, L., Weil, C., Shaw, J., 2011. IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res. Clin. Pract. 94, 311–321. https://doi.org/10.1016/j.diabres.2011.10.029

WHO | Diabetes programme [WWW Document], n.d. . WHO. URL http://www.who.int/diabetes/en/ (accessed 4.25.19).

Wu, Y., Li, H., Loos, R.J.F., Yu, Z., Ye, X., Chen, L., Pan, A., Hu, F.B., Lin, X., 2008. Common Variants in CDKAL1, CDKN2A/B, IGF2BP2, SLC30A8, and HHEX/IDE Genes Are Associated With Type 2 Diabetes and Impaired Fasting Glucose in a Chinese Han Population. Diabetes 57, 2834–2842. https://doi.org/10.2337/db08-0047

Zeggini, E., Weedon, M.N., Lindgren, C.M., Frayling, T.M., 2007. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 316, 1336–1341. https://doi.org/10.1126/science.1142364