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Effect of vitamin B12 on the isolates of *Candida albicans* from oropharyngeal candidiasis patients.

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ABSTRACT
Candida isolated obtained from the oropharyngeal of candidiasis patients was confirmed by microscopy, cultural characteristics and biochemical test and germ tube technique. The whole cell protein was isolated and SDS PAGE done to obtain molecular weight of the protein and anticandidial effect of vitamin B12 was proved by disc diffusion and well diffusion method . It was found that 30µg of vitamin B12 was effective against Candida albicans.

Key words : *Candida albicans, vitamin B12,oral thrush.*

INTRODUCTION

The opportunistic fungal pathogen *Candida albicans* is usually a harmless commensal organism found in the oral cavities of many healthy humans. However, in immune compromised patients *C. albicans* can cause a life threatening infection,(Lischewski and Ruhnke 1995). Human immunodeficiency virus works by weakening the body immune system, increase in retroviral replication and associated decline in immune defences, which render the patients particularly susceptible to oropharyngeal candidiasis , to the extent that the oral thrush is considered a strong indication of HIV associated immunodeficiency, (Enwuru *et al*,. 2008).

The identification of the species level of yeast cultured from various specimens has become increasingly necessary for clinical laboratories since *Candida albicans* is the yeast species most often isolated from clinical specimens and is frequently of clinical importance, (Yucesoy, 2001).

Generally the yeast identification procedure starts with germ tube test in clinical laboratories which is the rapid method to differentiate *C albicans* from other *Candida* species.This yeast is also confirmed as *Candida* by using Candida isolation agar test, methyl blue agar test, carbohydrate fermentation test and likewise the urease and nitrate tests are also done along with the SDS PAGE for finding molecular weight of the protein. An overgrowth of *Candida* and vitamin B12 deficiency is connected. Often when a person is suffering from *Candida* it prevents their bodies from producing and absorbing essential nutrients that the body need in order to remain fit and healthy. Often a person who is deficient in vitamin B12 will have a much lower red blood cell count than normal and this impact on the immune system, allowing the body to become more susceptible to suffer from different type of infection including Candida over growth.

So in this regard our study was aimed in isolating and characterizing *candida* from oral Candidiasis patients and assaying the whole cell protein along with the susceptibility study of vitamin B12 on the isolates.

SAMPLE COLLECTION

Samples were collected from the patients of different hospitals. From 10 patients 10 samples were collected.10 ml of sterile phosphate buffered saline (PBS) was swirled in the mouth for 1 min then expelled in to a container and this oral rinse sample was processed for Candida isolates. Samples were also taken by oral swab bring from the dorsal surface of tongue. These swabs were immediately immersed in PBS to prevent drying.

CULTURING OF ISOLATES

The oral rinses were centrifuged at 1,700 rpm for 10 min. The pellet was resuspended in 1ml of PBS, and vortexed for 30 seconds for 18 to 24 hour. And this was stored in SDA.

CONFIRMATION OF CANDIDA: Microscopic examination Gram staining of colony in media reveals round to oval budding yeast like cell or blastoconidia measuring 3.5 to 7 by 4 to 8 µm that retain crystal violet was considered to be *C.albicans* .

GERM TUBE TEST: The sample was added to serum and incubated at 35^{0}C for 2.5-3 hrs and observed under

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microscope Germ tubes were produced and the culture was identified as *Candida.*

METHYL BLUE SABOURAUD AGAR TEST: The media was prepared by adding .01% methyl blue dye in to SDA before autoclaving. Overnight colonies were then transferred to Petri dishes were incubated at 37^{0}c and results were evaluated after 24 hrs of incubation. The colonies which did not florescence were interpreted as being non *C. albicans* species while the colonies that fluoresced brightly were considered to be *C.albicans.*

The organisms were first spread on solid 2% agar medium which contained 1% bactopeptone, then incubated at 37^{0}C for 3 hrs and finally examined microscopically. A cover slip was placed over the organism at the time of inoculation. Nitrate and urease test were done for all the isolates. Glucose, sucrose, maltose, lactose fermentation tests were also done. Fermentation was described as the production of gas or bubble and possibly a colour change.

PREPARATION OF WHOLE CELL PROTEINS FOR SDS PAGE

For each culture, a loop full of overnight growth from SDA plate was suspended in 2ml sabouraud dextrose broth and incubated in a shaking incubator for 48 hrs (at 37^{0}C, 150 rpm). Sample were then transferred in to eppendroff tube and centrifuged for 3min at 12500 rpm and collected cell were washed 3 time with sterile distilled water .The washed cell were stirred after adding 25 µl SDS sample buffer. And the protein were denatured in boiling water for 5 minutes .The supernatant was centrifuged again for 3 min at 12100 rpm collected in an eppendroff tube and kept at 5^{0}C until electrophoresis were carried out.

SDS PAGE

Whole cell proteins were analysed by SDS- PAGE mainly to find out molecular weight protein. This method was done with 2 cm layer of 4% acryl amide staking gel and 10cm layer of 10% acryl amide separating gel. And the well was cut by the help of the comp and the isolated whole cell protein and the marker which containing the 14.3-97.4kda was kept in water bath for 5 min. Then 10 ml of sample and marker was loaded in well. Anode buffer (1.211gtris base/500distilled water) and cathode buffer (6.055tris base,8.96g tricine, 5 g SDS/500g of distilled water)was added. Then electrophoresis was done at 50volt.

ANTI CANDIDIAL EFFECT OF VITAMIN B12 ON ISOLATE: The Muller Hinton agar plate was prepared and the 24 hour culture of Candida was swabbed and vitamin B12 disc were placed on plates, and incubated at 37^{0}c for 24 hours**.** The Muller Hinton agar plate was prepared and the 24 hour culture of Candida was swabbed on it, wells were cut and 30 µg of vitamin B12 solution was put on wells of plates. And incubate at 37^{0}C for 24hours.

RESULTS AND DISCUSSION

A gram staining of a colony grown on routine primary media reveals round to ovel budding yeast cell of blasto conidia measuring 3.5-4.8 µm that retain crystal violet. After observing ander microscope the germ tube form of yeas were observed. and it was very thin and starting stage of another bud o fyeast. The colonies on methyl blue sabouraud agar were evaluated under U V lamp (wavelength 365 nm) and the isolates (*Candida albicans)* fluoresced brightly. The pearl formed colonies observed. Under microscope a cover slip placed over the organism time of inoculation Increased from 92.5-100%portion of isolates showed parallel sided germ tube. The isolate showed the nitrate reduction, urease production. And it has also proved that the organism had the ability to ferment the sugars like sucrose, glucose maltose, and lactose by producing gas. By these bio chemical tests it can be confirmed that the isolate from throat swab was *Candida albicans,* the results are shown in table 1.

The extracted whole cell protein was loaded in SDS PAGE to find out its molecular weight. And 5 protein bands were observed between the ranges of 20 k da to 40 k Da. This proved that the isolate have the whole cell of *Candid*a have minimum 5 proteins .which is responsible for producing Candidiasis

The zone of inhibition is mainly to find out the anti Candidial effect. Zone of inhibition was observed in MHA plates around the Candidial growth by agar well diffusion and agar disc diffusion. After incubation diameter of the zone was measured. In the disc diffusion method the zone was in between 2-5 5cm and incase of well diffusion it was 3.5-5 cm, the data are shown in table no 2.

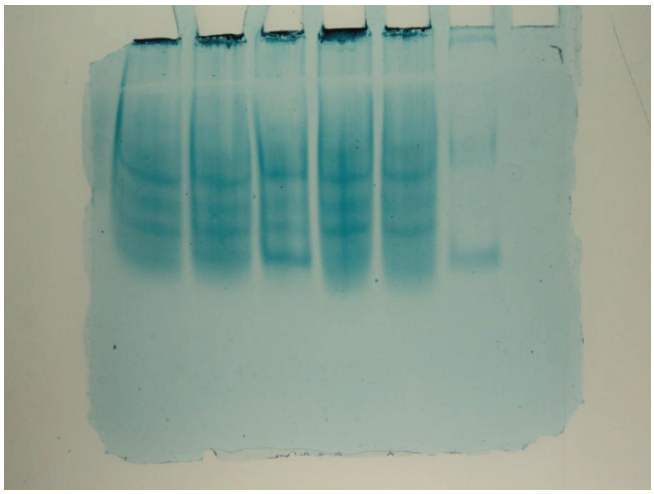
TABLE 1 : Biochemical parameters of *Candida albicans*

SNO	BIOCHEMICAL TEST	RESULT
1	NITRATE REDUCTION	POSITIVE
2	UREASEPRODUCTION	POSITIVE
3	SUGAR FERMENTATION	
3.1	GLUCOSE FERMENTATION	ACID WITH GAS
3.2	SUCROSE FERMENTATION	ACID WITH GAS
3.3	MALTOSE FERMENTATION	ACID WITH GAS
3.4	LACTOSE FERMENTATION	ACID WITH GAS

The isolates were screened for the susceptibility to vitamin b12 by agar well diffusion and disc diffusion method. Results are tabulated in table 2

TABLE 2 : Showing the zone of inhibition of the anti candidal effect

SNO		DIFFUSION METHOD ZONE SIZE(DIAMETER)	
	CULTURE	WELL DIFFUSION	DISC DIFFUSION
1	Ca1	4 cm	2 cm
2	Ca2	5 cm	3 cm
3	Ca3	3.5 cm	2 cm
4	Ca4	5.5 cm	3 cm
5	Ca5	4.3 cm	3 cm
6	Ca6	4 cm	2 cm
7	Ca7	3 cm	2 cm
8	Ca8	5 cm	3 cm
9	Ca9	4cm	2.5 cm
10	Ca10	4.5 cm	2.4 cm



SDS PAGE OF WHOLE CELL PROTEIN OF C*andida albicans*

Candida is an opportunistic fungus, which has thin walled yeast. In the present study *Candida albicance* were isolated from different hospitals and it was done by the oral rinsing method and tongue swab method (*Ponrs*gwet *et al*., 2004). The yeast identification procedure started with germ tube test. Although this is a rapid test it may lead to both false positive and false negative results. When the yeast cannot be named with these results further test such as carbohydrate fermentation such as automated identification

procedure were perfomed. Several culture media containing flurogenic and chromogenic substrate specific for Candida were used (Yu*cosoy et al*., 2000). The whole cell protein was isolated from the organism to find out the protein present in the *Candida* and the SDS PAGE also helped to find out the molecular weight of these proteins. It was confirmed as it had more than 5 proteins at the molecular range of 20-40 KDa (Ozlem and Altinar *et al.,* 1999).

The zone of inhibition of *Candida albicance* showed that it had anti candidial effect which can be affected by vitamin B12 deficiency. This is yeast that grows in the body, most notably in the vaginal area and in the intestines. In most cases, it is kept under control by the body's immune system. However, when there is a lack of B12 the immune function is impaired and *Candida* can grow out of control. When this happens, a person might notice headaches, mental fog, tiredness, sinus issues, mood swings, loss of concentration, and even a craving for sweet foods. In order to treat *Candida*, the deficiency of vitamin B12 needs to be taken care of first. When the proper balance of B12 is restored, *Candida* can then be kept in check after a course of treatment it is reverted back to its normal state within the body (Symms , 2005)

Candida is an opportunistic pathogen, it usually causes oropharyngeal candiasis in aids patients, and this candidial protein also causes the infection in mouth and pharyngitis.The candidial culture which is collected from various hospitals and isolated by different methods was confirmed by method as described above. It has been proved that vitamin B12 decreases the growth of the *Candida,* so we can conclude that the vitamin B12 can be used for oropharyngeal candida treatment.

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