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Perspective

Qualitative and Quantitative Assessment of Candida Species in Type-2 Diabeties with and without Smoking

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Introduction

The term diabetes mellitus is used to identify a group of disorders, characterized by elevated levels of glucose, in the blood. It is estimated that there are about 170 million people with diabetes mellitus in the world accounting 7% of the total world adult population. These Candida species were discovered by a botanist Christine Marine Berkhout in the year 1923. The prevalence of microorganism in diabetic patients varies from 18-80% [1,2]. Candida is a part of normal microbial flora. It becomes pathogenous when oral microbial flora balance is altered. Smoking is associated with variety of changes in the oral cavity. Literature reveals that candida carriage in the tobacco smokers is higher than in non-smokers. Smoking has effect on saliva, oral commensal bacteria, fungi, and mainly candida [3,4].

Many studies focusing on the association of Candida in diabetics and smokers were evident, though the comparison and correlation of Candidal species among diabetic patients and diabetic smokers were sparse. Hence the aim of the present study focused on quantifying the Candidal organism in type-2 diabetics with and without smoking. Also efforts were made to identify the association of Candidal species in above mentioned study groups.

Methodology

Materials and methods

Study includes total of 225 cases:

Group-I: Controls (75 cases)

Group-II: Diabetics (75 cases)

Group-III: Diabetic smokers (75 cases)

Inclusion criteria

- Newly diagnosed type-2 diabetes patients before initiation of standard prescribed medication.
- Male population with the age limit of 40-60 years.
- Individuals with diabetes and smoking habit of minimum 5-10 cigarettes/day from past two years.

For controls

- Age and gender matched healthy individuals from similar population group after Random blood glucose estimation.

Exclusion criteria

- Type-1 diabetic patients if any will be conformed based on c-peptide test.
- Patients with oral prosthesis.
- Patients receiving Radiotherapy
- Patients under long-term local and systemic drug therapy.

Preparation and collection of oral rinse

Sterile phosphate saline buffer is prepared by dispensing 10.7gm of phosphated buffered saline powder in 1000ml of distilled water (pH:7.4). The prepared solution is autoclaved and dispensed into 3ml sterile containers for use as oral rinse. Patients were asked to rinse their mouth using 5ml of phosphate buffered saline for 2min and collect in a sterile container.

Preparation and specimen culture of SDA plates (Samaranayake et al.)

6.5gm of sabouraud dextrose agar (HI MEDIA-M063) is dispensed in 100ml distilled water (pH:5.6), Supplemented with chloramphenicol 10mg/ml. the suspension is autoclaved at 121^{0}C for 15min at 15lbs pressure. The solution is cooled and mixed well and pours into petri plates. Culture inoculation was done using a loop of the sediment in a wavy pattern and incubated for 3 days at 37^{0}C with a regular follow up. Cream colored shiny oval colonies were evident after 48 hours representative of Candida. The colony forming units (CFUs) were counted using Lapiz digital colony counter for each patient.

Preparation of specimen culture of CHROMagar plates

4.2gm of CHROMagar is dispensed in 100ml distilled water (pH:5.6), supplemented with chloramphenicol 10mg/dl. The suspension is heated to dissolve the medium completely. Do not autoclave. Cool to 45-50^{0}C. Mix well and pour into sterile petri plates. Culture inoculation was done using a loop of the sediment in a wavy pattern and incubated for 3 days at 37^{0}C with a regular follow.

Sample collection

Figure 1 and Table 1.

Results

The study was cross sectional in nature which comprised of 225 subjects between age groups of 40-60 yrs. These subjects were divided

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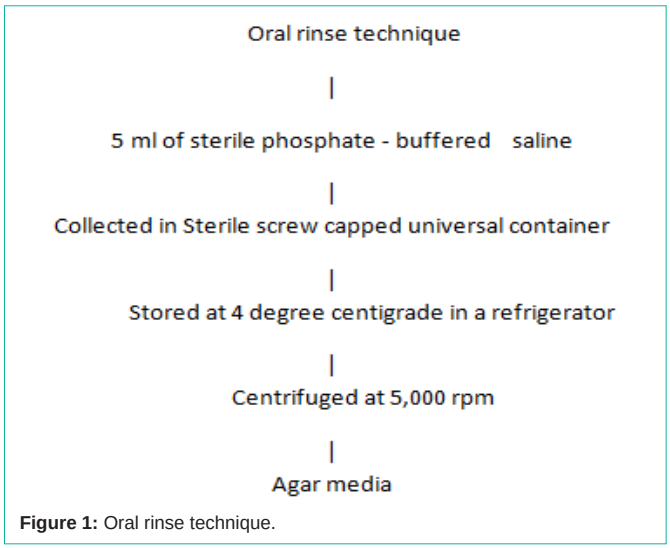

Figure 1: Oral rinse technique.

Table 1: Sample Collections.

Species	Colour on HiChrome Agar
Candida albicans	light green
Candida tropicalis	Purple halo in agar, dark blue colour
Candida parapsilosis	pale colour
Candida glabreta	Dark pink
Candida dublineinsis	Dark green

Table 2: Age association between 3 groups.

Age	Controls	Diabetic patients	Diabetic smokers
40-50 yrs	39 (52%)	41 (54.6%)	43 (57.3%)
50-60 yrs	36 (48%)	34 (45.3%)	32 (42.7%)
Total	75 (100%)	75 (100%)	75 (100%)

Table 3: Total Candidal colony forming units in different study groups.

SDA	Candida colonies
Controls	1506
Diabetes	3131
Diabetic Smokers	5353

into study groups which include 75 controls, 75 diabetic patients and 75 diabetic smokers (Table 2).

Group-1: Controls

Group-2: Diabetic patients

Group-3: Diabetic Smokers

Quantitative assessment of Candidal colonies by using Sabouraud Dextrose Agar media

In Sabouraud's Dextrose media, One way ANOVA test revealed significant interaction between groups (P = 0.000) with increase in type -2 diabetic smokers (P = 0.000). In chrome agar, Two way ANOVA revealed significant increase in *candida albicans* (P = 0.000) in type-2 diabetic smokers with significant interaction within the groups (P = 0.000) tests revealed significant Candidal colonies in the decreasing order of diabetic smokers, diabetes and controls (Table 3).

Qualitative assessment of Candidal species by using CHROMagar media

CHROMagar: TWO ways ANOVA performed.

In CHROMagar-between groups and species, between groups, between species were analyzed depicts that there is significant difference between groups and increased levels of Candidal colonies in diabetic smokers with increased levels of Candida albicans species (P-value: 0.000) depicts that there is increased levels of Candidal colonies in diabetic smokers followed by diabetes and control groups (Table 4 and 5).

Discussion

Diabetes is rapidly becoming a major public health problem worldwide. Diabetes mellitus is one most common endocrine metabolic disorder characterized by inability of the body cells to utilize glucose. WHO described it as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism resulting in defects in insulin secretion, insulin action or both. It is considered as a disorder of abnormal carbohydrate metabolism that results in acute and chronic complications due to relative lack of insulin [4,5].

Type-2 diabetes is the most common type of diabetes comprising 90% of people with diabetes around the world. It is characterized by slow onset of symptoms usually often 40 years of age and now taking its place as one of the main threats to human health in the 21st century. According to the diabetes atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025. The association of Candida infection and increased glucose levels in diabetes has been creating a scope of interest now a days [6-9].

The study consisted of 225 male individuals (75 controls, 75 diabetics and 75 diabetic smokers) with age range between 40-60 years. The mean age for controls, diabetic and diabetic smokers were 49.36, 47.27 and 49.45 respectively. These findings were in similarity with earlier population studies conducted in a south Indian population by Rao PV et al. in 35,000 individuals aged above 40 years. Adaptation of sedentary behavior, reduced physical activity and diet habits like fast food cultures with age was suggested as a reason for this variation, increase in the prevalence of type 2 diabetes may also result due to migration, which brings marked social and cultural changes. Misra et al. reported that migration from rural areas to urban metropolitans in

Table 4: Assessment of Candidal species in different study groups by using CHROMagar.

	Diabetic	Diabetic Smokers	Controls
Candida glabrata	19	14	0
Candida albicans	1666	3711	952
Candida tropicalis	403	1216	26
Candida krusei	19	345	0
Candida parapsilosis	0	201	0

Table 5: Candida albicans levels in 3 different groups using CHROMagar.

	Controls	Diabetics	Diabetic smokers
Candida albicans	952	1666	3711

India led to obesity and glucose intolerance [10-12].

The prevalence of oral Candida infections among Andhra Pradesh state representive group in type-2 diabetic patients and type-2 diabetic smokers is consistent with numerous previous studies, which have shown that diabetes mellitus is a major predisposing factor to symptomatic oral candidiasis. This is also in agreement with numerous previous studies, which have all indicated that diabetes mellitus enhances Candida colonization and proliferation. In addition to diabetes mellitus, the prevalence of oral Candida infections is influenced primarily by smoking. It is also clear from the findings presented in this study that normal individuals whether diabetics or not, are less prone to *C. albicans* colonization. Tapper-Jones et al. have shown that 42% of healthy nondiabetics harbour *C. albicans* in their mouths compared to 60% of diabetics [13,14]. Yarahmadi et al. have suggested that 16.2% of the controls and 40.2% of the diabetics carry *C. albicans* in the mouth [15].

Conclusion

Present study showed significant increase in the number of candidal colonies in Type-2 diabetic smokers. Among species, study showed increased *candida albicans* in diabetic smokers indicative of synergism of smoking and diabetes in the growth of candida.

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