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RESEARCH ARTICLE

ASSOCIATION OF ORAL CANDIDA CARRIAGE IN PREDIABETIC SMOKERS, NON-SMOKERS AND CONTROLS

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ABSTRACT

The prevalence of diabetes and pre-diabetes has reached epidemic proportions. Worldwide, the number of people with pre-diabetes (impaired glucose tolerance) in the age group 20–79 was 308 million in 2007, and is expected to increase to 418 million by the year 2025.

Aims and Objective: To evaluate and compare the association of oral candida amongst 1. Smoker prediabetic. 2. Non-smoker prediabetic. 3. Controls.

Materials and Methods: The sample size included 60 patients i.e. 20 smoker prediabetic, 20 nonsmoker prediabetic and 20 age and sex matched controls. Fasting blood sugar levels were obtained using routine haemogram. Oral Candida samples collected from saliva. At 37 ^{o} C Candida strains were cultured in Sabouraud dextrose agar and quantified**.**

Results: Oral *Candida albicans* carriage was significantly higher in prediabetic smokers (60.6%) and the prediabetic non-smokers (23.3%) compared with controls (16.7%). There was a statistically significant difference observed amongst three groups in the mean CFU value.

Conclusion: The prevalence of oral *Candida* carriage was significantly higher in prediabetic smokers than prediabetic non-smokers compared to controls. Since *Candida* species may cause opportunistic infections in immune-suppressed patients, additional attention should be paid to usage of tobacco particularly in patients with immune-suppressive disorders.

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INTRODUCTION

Prediabetes, a state of impaired glucose tolerance (IGT), is characterized by IGT (140 to 199 mg/dL), impaired fasting glucose (100 to 125 mg/dL), or both. In addition, Individuals with HbA1c levels between 5.5% and 6.4% are categorized as individuals with prediabetes. The World Health Organization (WHO) has defined prediabetes as a state of intermediate hyperglycemia characterized by, impaired fasting plasma glucose level (100 to 125 mg/dL). The worldwide prevalence of prediabetes in 2010 was estimated to be 343 million (7.8%). In India more than 10 million cases per year. International Diabetes Federation projects an increase in prevalence of prediabetes to 471 million globally by 2025 (Susanne Anderson *et al*., 2008). Tobacco smoking is a significant risk factor for an increased oral Candida carriage. Because xerostomia is a common manifestation in patients with chronic hyperglycemia,it is assumed that oral Candida carriage is high in prediabetes tobacco users compared with prediabetic non-tobacco user and healthy controls. We therefore hypothesized that oralCandida carriage would be increased in prediabetes tobacco users than prediabetic non-tobacco user and controls. Toour knowledge from indexed literature, this hypothesishad not been tested so far.

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INCLUSION CRITERIA

- Only individuals with Medically diagnosed Prediabetes (FBGL, 100-125 mg/dl)

- Tobacco smoking or exclusive areca nut and gutka chewingfor past 5 years.

EXCLUSION CRITERIA

- Use of antibiotics, antifungal agents, steroids, or nonsteroidal anti-inflammatory drugs within the past 3 months.
- Those who were wearing partial or complete dentures.
- Self-reported systemic diseases, including type 1 and type 2 diabetes mellitus, hepatitis B, hepatitis C, and infection with HIV or AIDS.

MATERIALS AND METHODS

Study sample size included 60 patients between the age group of 30-70 years. Group A 20 prediabetic smokers, Group B 20 prediabetic non-smokers and group C 20 controls. It was mandatory for all study participants to have read and signed the consent form before being included in this study. Patients with prediabetes were recruited from the dental OPD with family history of diabetes mellitus and clinically diagnosed as generalized chronic periodontitis. Control participants were self-reporting as not prediabetic were recruited from area near the hospital. All participants were invited to the hospital in the early morning hours (in a fasting state) for FBGL measurement and collection of oral yeast and unstimulated whole saliva (UWS) samples.

Fasting Blood Glucose Levels

A digital glucometer (Accu-Chek Activ, Roche Diagnostics, and Mannheim, Germany) was used to measure the FBGL. Depending on the glycemic levels, patients with prediabetes were divided into 2 subgroups as follows: Group A, smoker prediabetes with FBGL between 100 and 125 mg/dL, and group B, nonsmoker prediabetes with FBGL between 100 and 125 mg/dL. Self-reported systemically healthy individuals (FBGL, 70 to <100 mg/dL) were categorized as controls (group C).

Collection of UWS samples: To collect the UWS samples, participants were seated comfortably in a chair in a "coachman" position and requested to spit (without swallowing) into a gauged measuring cylinder for five continuous minutes.

Collection of oral yeast samples: Oral Candida samples were collected as saliva described previously. Immediately after sampling, the samples were returned to the containment tube to avoid contamination. At 37 C, Candida strains were cultured in Sabouraud dextrose agar (fig 1) (Becton, Dickinson and Company, Sparks, MD, USA) to quantify the colony-forming units in the oral cavities of individuals with prediabetes smokers, nonsmokers and controls. After 24 hours, all cultures were inspected, and monitoring continued until 2 days of incubation for yeast growth, following which they were subjected to speciation. Yeast colonies were smeared and gram stained viewed under 100x magnification. (fig2) Germ tube test or Reynolds-braude phenomenon is the rapid and confirmatory test for *Candida albicans.* It was done by incubating a loop of yeast culture in 0.5 ml of human serum at 37 c for 2 hours. A drop of the suspension was placed on a slide using a Pasteur pipette and covered with a coverslip. Examined at 40X for production of germ tubes (long tube-like projections extending out from the yeast cells) (fig3)

STATISTICAL ANALYSIS

The mean comparison among groups (A, B, and C) was carried out with ANOVA. For multiple comparisons, the Bonferroni post hoc test was used. Level of significance was set at $P < .05$. Data were analyzed using SPSS software version 20.

Characteristics of the study cohort

Twenty patients with prediabetes smokers (20 patients [16 males and 4 females] in group A) and prediabetic non-smokers 20 patients [9 males and 11 females] in group B and 20 controls group C (9 males and 11 females) were included for study. There was a statistically significant difference in the distribution of sex among three groups, $p<0.05$. More number of male present in the smokers group (Table 1). There was a statistically significant difference in the distribution of age among three groups, $p<0.05$. Cases in the age of 51-60 years were present in the prediabetic smokers group A. Cases in the age of 41-50 years were present in the prediabetic non-smokers group B.(Table 2). No significant difference found in the distribution of age between male and female. The mean FBGL was significantly higher among the population with prediabetes smokers in group A (114.05 mg/L) and prediabetic non-smokers in group B (113.05 mg/dL) than among individuals in the control group (84.85 mg/dL) ($P < .05$), respectively. Mean FBGL was significantly higher among patients with prediabetes in group A (114.05 mg/dL) than in group C (84.85 mg/dL) ($P<.05$) (Table 3).

Oral Candida carriage

Oral C albicans carriage was significantly higher in the population with prediabetes smokers in group A (n =20) (60.6%) and the patients with prediabetes non-smokers in group B (23.3%) compared with group C (n =20) (16.7%) There was a statistically significant difference observed among three groups in the mean CFU value using ANOVA, $F=69.39$, $p<0.001$(Table IV).The analysis of variance using post hoc by Bonferroni showed that there was significant mean difference observed between control group and the PS group, $p<0.001$ and PNS and PS group $p<0.001$. There was no significant difference observed control and PNS groups $p>0.05$. (Table 5).

Table 1.

Gender	Groups			Chi-square	P value
	Control	PS	PNS		
Male	9	16	9	6.65	0.03*
Female	11	4	11		

*Significant p<0.05

Table 2.

Age in years	Groups				
	Control	PS	PNS	Chi-square	P value
≤30	4	1	1		
31-40	6	3	3		
41-50	6	4	12	17.72	0.02*
51-60	4	10	2		
61-70	0	2	2		

*Significant p<0.05

Table 3.

Groups	N	Mean FBGL	SD	F	P value
Control	20	84.85	9.34		
PS	20	113.05	7.68		
PNS	20	114.0	5.68	92.15	0.000**

*Significant p<0.001

Table 4.

Groups	N	Mean FBGL	SD	F	P value
Control	20	84.85	9.34		
PS	20	113.05	7.68		
PNS	20	114.0	5.68	92.15	0.000**

*Significant p<0.001

Table 5.

Groups	N	Mean CFU	SD	F	P value
Control	20	450.00	201.31		
PS	20	1705.00	549.14		
PNS	20	655.00	223.55	69.38	0.000**

*Significant p<0.001

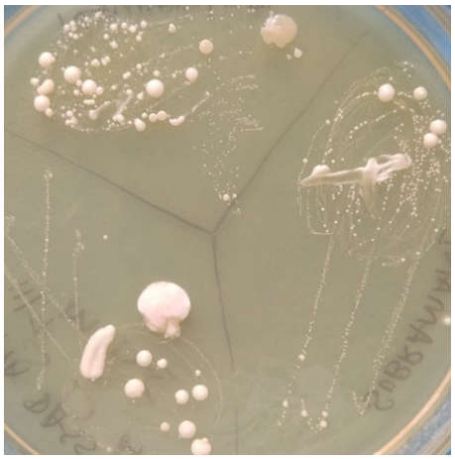

Fig. 1. Yeast colonies in Sabouraud dextrose agar at 37 c after 48 hours

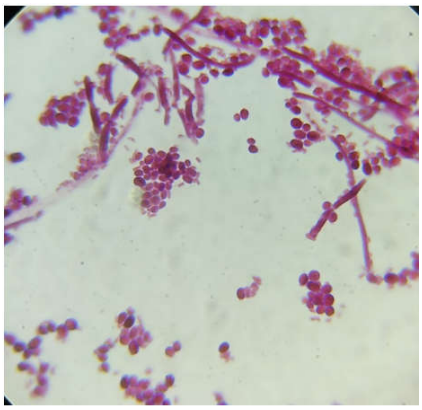

Fig. 2. Dimorphic candida albicans under 100x magnification

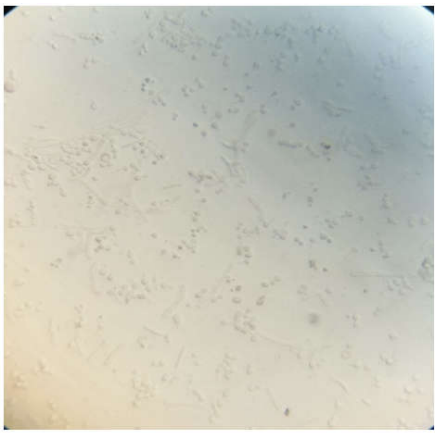

Fig 3 Germ tube formation of candida albicans under 40x magnification

DISCUSSION

To our knowledge from indexed literature, this is the first study in which oral Candida carriage was investigated in patients with prediabetes with particular emphasis on glycemic status and tobacco usage. In general, the population with prediabetes smokers and non-smokers investigated in the present study was hyperglycemic (FBGL, 114.03 mg/d and 113.05 mg/dl), which is a possible explanation for the increased oral C albicans carriage in patients with prediabetes (n =40) compared with healthy controls (20 individuals in group C). Our findings are in accordance with those of earlier studies (Fawad Javed, 2009; Sultan Al Mubaraka *et al*., 2013; Ramon Felipe Fernandez Martinez *et al*., 2013), in which oral Candida carriage was reported to be increased in patients with poorly controlled type 2 diabetes as compared with controls. In the literature, there are several hypotheses why tobacco consumption enhances *Candida* colonization. Tobacco usage leads to an increase in thickness of epithelial keratinised layer, decrease in levels of salivary immunoglobulin A, and suppression in functions of polymorphonuclear leukocytes, thus facilitating the proliferation of *Candida* species.

It is also hypothesized that cigarette smoke enhances adhesion, growth and biofilm formation of C albicans. Another hypothesis is that tobacco content (such as nicotine, nitrosoprolin, nitrosodietheinalamine, and polycyclicaroma-tic hydrocarbons and polonium) causes a media which facilitates the proliferation of *Candida* species. Moreover, some other hypotheses propose that nicotine in tobacco causes functional and structural alterations in keratinocytes and other components of tobacco lead to decrease in epithelial cells and antifungal activity. Most personswith prediabetes (impaired glucose tolerance or impaired fasting glucose) are overweight, and obesityworsens themetabolicand physiologic abnormalities associated with this condition (Everardo Albuquerque Menezes *et al*., 2007). Prediabetes is an important risk factor for the development of type 2 diabetes. DM has been considered a predisposing factor for candidiasis. In this disorder there is a decrease of the defensive capacity of polymorphonuclear neutrophils (PMN) and T-lymphocytes related to hyperglycemia, generating a favorable environment for the reproduction of species of Candida. The pathogenesis of *Candida* species is related to a combination of factors that contribute to its virulent. The production of extracellular enzymes, Such as proteinase and phospholipase, as one of the main Mechanisms of virulence (Norris *et al*., 2005).

In the present study, we found that 60.6% of prediabetic smokers, 23.3% of the prediabetic nonsmokers and 16.7% of the controls were carriers of oral *Candida*. It was determined that the prevalence of *Candida* carriage was significantly higher among prediabetic smokers and prediabetic nonsmokers compared to the controls. Prediabetic smokers were present more in age group of 51-60 years which speculates that elderly individuals placing betel quid in the mouth for prolonged durations harbor high percentages of mixed Candida species that could make them more susceptible to oral infections compared to younger betel quid-chewers and controls. In a study by Darwazeh *et al*. it was reported that the rate of *Candida* carriage was 84% in smokers and 74% in the non-smokers. In the literature, while some studies revealed a significantly higher rate of *Candida* carriage in the smokers compared with non- smokers, others showed similar rates between smokers and non-smokers (Darwazeh *et al*., 2016;

Hamit Sirri Keten *et al*., 2015). Keten *et al*. reported that 54% of Maras powder users were *Candida* carriers while 22% of the non-users were carriers. *Candida* carriage among betel quid (a kind of smokeless tobacco) users and non-users showed the prevalence of *Candida* carriage to be 73.4% in the users and 61% in the non-users (Fawad Javed *et al*., 2013). The distinctness of results of the studies may stem from differences in study populations (age, gender, race, and systemic diseases), content of tobacco products and the ways of using the product, diet, and genetic characteristics. It was shown that poor glycemic control, old age and tobacco usage habit increased *Candida* carriage. There are a few limitations of the present study that we address. First, association of oral and tongue lesion to glycemic levels was not performed, and this would have been useful for better significance of the outcome. Second, categorization of the individuals with prediabetes and controls was based on measurement of FBGL levels. It is known that the oral glucose tolerance test (OGTT) isa valuable and reliable tool for monitoring hyperglycemia (Ouchi *et al*., 2012); therefore, it is highly recommended that OGTT should be considered as a critical parameter in future studies dealing with glycemic status in patients with diabetes and in undiagnosed individuals. Third, most of our study participants were men. It has been reported that oral Candida carriage is significantly higher in women with type 2 diabetes compared with men with type 2 diabetes (Fawad Javed *et al*., 2009). Thus, further studies are needed to assess the limitations of the present study.

Conclusion

Within the limits of the present investigation it is concluded that the prevalence of oral *Candida* carriage was significantly higher in prediabetic smokers than prediabetic non-smokers compared to controls. Since *Candida* species may cause opportunistic infections in immune-suppressed patients, additional attention should be paid to usage of tobacco particularly in patients with immune-suppressive disorders. Furthermore, appropriate precautions about restriction and cessation of tobacco products are of great importance for preventive and therapeutic health services.

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