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EVALUATION OF THE PREVALENCE OF *CANDIDA ALBICANS* INFECTION IN PATIENTS WITH ORAL SUB MUCOUS FIBROSIS IN COMPARISON WITH HEALTHY INDIVIDUALS

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Abstract: To evaluate the prevalence of *Candida albicans* infection in patients with Oral Sub Mucous Fibrosis (OSMF) in comparison with healthy individuals. The study included 60 patients who were divided into three groups (n=20). Group-I: Controls, group-II: Smokers, group-III: Smokers with Oral Submucous Fibrosis. Demographic data and salivary samples were collected from all the groups. The isolated organisms were counted and the number was compared among the groups. P value was found to be less than 0.05, hence the study was considered to be statistically significant. The number of *Candida albicans* colonies was observed to be higher in group-III compared to groups-I and II. Patients having OSMF with smoking habit showed more number of colonies of *Candida albicans*. These values were found to be statistically significant. Smokers with OSMF (group–III) showed higher count of *C. albicans*. Further studies are required to find out the correlation between *Candida albicans* infection and smoking.

Key words: Oral submucous fibrosis, *C. albicans*, Gram staining, smoking.

INTRODUCTION

Oral candidacies (also known as oral candidosis, oral thrush, oropharyngeal candidiasis, moniliasis, candidal stomatitis) are a mycosis (fungal infection) of candida species on the mucous membrane of the mouth. *Candida albicans* is the most commonly implicated organism in this condition.

Candida albicans is carried in the mouths of about 50% of the world's population as a normal component of the oral microbiota, but is kept in check by our immune system. This candidal carriage state is not considered a disease, but when *Candida* species become pathogenic and invade the host tissues, oral candidiasis can occur. This change usually constitutes an opportunistic infection of normally harmless micro-organisms because of local (i.e., mucosal) or systemic factors altering host immunity. Thus, depending on the host defense mechanisms or local oral microenvironment, *Candida* can transform from a harmless commensal to the pathogenic organism causing oral mucosal infection (4, 5). These opportunistic fungal pathogens may colonize, invade and induce lesions in any part of the oral cavity in immunocompromised individuals (16).

Candidal infection together with co-factors such as vitamin deficiency and generalized immune suppression may play a contributory role and may induce epithelial atypia and dysplasia leading to malignant change through the release of chemical carcinogens like nitrosamine compounds (2, 3). Strains with high nitrosation potential were isolated from lesions with more advanced precancerous changes. The yeast cells in such cases extend from the mucosal surface to the deeper epithelial cell layers representing transport and deposition of precursors like nitrosamines to the deeper layers. This showed that certain strains of *C. albicans* play a key role in the development of dysplasia (8).

Oral submucous fibrosis is an insidious chronic disease affecting any part of the oral cavity and sometimes pharynx. It is constantly associated with juxta-epithelial inflammatory reaction followed by fibro-elastic change in the lamina propria and epithelial atrophy; which leads to stiffness of the oral mucosa, causing trismus and inability to eat (9).

A multifactorial model for the pathogenesis of OSMF is postulated. Tobacco, lime, betel quid, iron and nutritional deficiencies, chronic candidiasis, genetic abnormalities, viral infections, autoimmunity etc., are considered to have either direct effect in causing OSMF or an indirect effect by intervening the immune system which is compromised in OSMF (10). A considerable proportion of oral squamous cell carcinomas develop from preexisting potentially malignant disorders of the oral cavity (6). World Health Organization (WHO) in 2007 proposed the term potentially malignant oral disorders (PMD) for precancerous lesions and conditions (7).

Oral Sub Mucous Fibrosis (OSMF) is a high risk precancerous condition which favors the colonization of *Candida*. Mucosal alterations due to the underlying disease process or betel quid chewing, coupled with other factors, might lead to candidal colonization, even in the absence of clinically-related mycotic manifestations (1). Epithelial atrophy is one of the cardinal histopathologic features of OSMF. Presence of Candida in the mouth together with epithelial changes may predispose to candidal infection. Decreased mouth opening which is another characteristic feature of OSMF may also predispose an individual to candidal growth, and this *Candida* can further predispose the mucosa for malignant transformation through the process of nitrosation (2, 3). This study was conducted to evaluate the prevalence of candidal infection in Smokers with Oral Submucous Fibrosis.

MATERIALS AND METHODS

The study included a total of 60 male patients. All the patients selected were those coming to the outpatient department. The selected patients were divided into three groups, each containing 20 patients.

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Study groups

Group-I: Control group
Group-II: Smokers
Group-III: Smokers with Oral Sub mucous fibrosis

The clinical details of all the patients were obtained. Patients with OSMF with other systemic diseases were excluded from the study.

Collection of samples

Oral yeast colonization was assessed with the concentrated oral rinse technique as described by Samaranayake *et al.,* (19). The subjects were instructed not to eat and drink 2h prior to the sample collection.

Subjects belonging to all the three groups were asked to rinse their mouth with 10ml of phosphate buffered saline (PBS) which was held in the mouth for 2min prior to collection in a sterile container. The samples were thus collected and immediately transported to the central laboratory. Each rinse was centrifuged at 2,500 RPM/10 min, the supernatant was removed, and the deposit was resuspended in PBS. A known volume, usually 100µL of this concentrate was inoculated onto Sabouraud's dextrose agar (SDA). Colony forming units resembling yeast growth were removed from the plates and processed further for identification using Gram staining, a germ tube test, chlamydospore formation and sugar assimilation tests. After 48 hrs of incubation at 37°C, growth was assessed by enumeration of colonies and expressed as candidal colony forming units per mL (cfu mL^{-1}) of rinse [16]. To differentiate between *Candida albicans* and other species, growth was assessed at 45 °C on modified Sabouraud's glucose agar (SGA). The colonies were counted and expressed in colony forming units per mL (CFU/mL) of the collected sample.

Statistical analysis

Statistical Package for Social Sciences (SPSS 16.0 version) was used for analysis. One way ANOVA (Post hoc) followed by Dunnet t test was applied to find statistical significance among the groups. P<0.05 considered statistically significant at 95% confidence interval.

RESULTS

In Group-III, 80% of patients showed the presence of *C. albicans,* which was statistically significant compared to the other groups (Table-1). In the degree of prevalence also, group-III showed (834.93±156.34), group-II (698.32±117.03) followed by group-I (156.78±120.96). The difference in values among the groups was found to be statistically significant. Smokers with OSMF (Group-III patients) had more colony forming units compared to the other groups (Table-2, Graph-1).

Table 1: Prevalence of *Candida albicans* among the groups

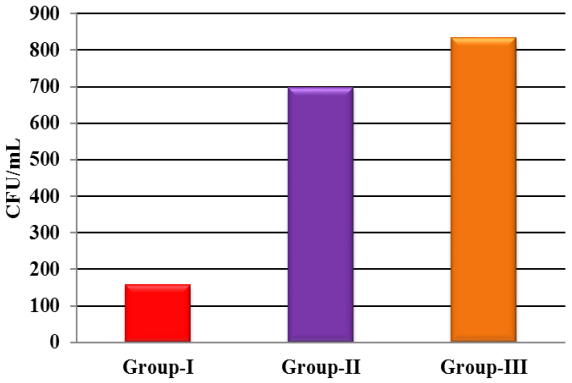
Groups	Health condition	Number	Percentage (%)
Group-I	Control group	3	15.00
Group-II	Smokers	10*	50.00
Group-III	Smokers with OSMF	16*,#	80.00

(*P<0.05 significant when group-I was compared with the other groups, #P<0.05 significant when group-II was compared with the other groups)

Table 2: Comparison of prevalence of *Candida albicans* among the groups

Groups	Prevalence of *Candida albicans* (CFU/mL) (MEAN±SD)
Group-I	156.78±120.96
Group-II	698.32±117.03*
Group-III	834.93±156.34*,#

(*P<0.05 significant when group-I was compared with the other groups, #P<0.05 significant when group-II was compared with the other groups)



Graph 1: Comparison of prevalence of C. *albicans* among the groups

DISCUSSION

Candida albicans is the naturally occurring fungus in our body. The overgrowth of *Candida albicans* leads to infection called candidiasis. This can attack the mucous membrane, oral cavity, vagina, blood stream, gastrointestinal tract and lymphatic system. The major symptoms are white patches, pruitus and curd like discharge. Several agents and the use of some of the drugs can promote candidal infection. The agents that suppress the immune system are the ones that increase the severity of infection. Amit Chattopadhya *et al.,* studied smoking as a risk factor for oral candidiasis in HIV infected adults. The results of the study showed that HIV patients with smoking habit are more prone to candidal infection compared to the other groups [11]. Smoking has both long and short term effects on the oral cavity. Cigarette smokers had a significantly increased carrier rate, compared with non-smokers (18). It increases the proinflammatory factors, oxidative stress and depresses the immune mechanism of the oral cavity. These effects of smoking favor candidal infection. Tobacco smoke have been found to affect both cell-mediated immunity and humoral immunity. Research on gingival crevicular fluid has demonstrated that there are lower levels of cytokines, enzymes and possibly polymorphonuclear cells in smokers (12).

The frequency of isolation of *C. albicans* and its mucosal density per unit area, as measured by imprint culture, was highest on the dorsum of the tongue, particularly the posterior half. It has been found that Candida is not uniformly distributed throughout the mouth .The tongue is the primary oral reservoir for the fungus, from which the rest of the oral mucosa, plaque-coated surfaces of the teeth and the saliva may become secondarily colonized in a proportion of carriers (18).

Consumption of tobacco in any manner (smoking, chewing) increases the colonization of candida due to increased epithelial keratinization, decreased salivary immunoglobulin A and decreased function of leukocytes [13, 14]. The increased susceptibility of cigarette smokers to infections reflects multifunctional alteration of their innate and adaptive immune responses (14). Eichel *et al.,* reported that a single cigarette provided enough toxic material that completely inhibited the function of oral salivary neutrophils *in situ*. Reduced phagocytic activity of neutrophils was also reported in smokers which could be responsible for reduction in the defense mechanism of the gingival tissues against bacterial attack (14).

The studies of Abdelhabib Semlali *et al.,* clearly showed that smoking increases *C. albicans* adhesion, growth and biofilm formation. They demonstrated that CSC-exposed (Cigarette smoke condensate –exposed) *C. albicans* expressed high levels of *EAP1*, *HWP1* and *SAP2* (secreted aspartic protease 2) mRNA and that this gene expression increased with increasing concentrations of CSC [15].

This study revealed that smokers had more number of candidal colonies compared to the control group. Studies by Anila K *et al.,* showed that the degree of prevalence of Candida was greater in OSMF patients compared to the control group [16]. Studies by Ariyawardana *et al.,* showed that the prevalence of *Candida albicans* species in OMFS patients was 63.6% compared to healthy individuals [17]. As the oral mucosa is compromised in OSMF, it can be concluded that the presence of *Candida albicans* may predispose the individual to infection. In this study, Smokers with OSMF showed significant increase in the number of *Candida albicans* compared to the other groups.

CONCLUSION

Smokers with Oral Sub Mucous Fibrosis are more easily prone to develop *Candida albicans* infection compared to the other groups. However, more studies are required in this direction to find out the correlation between smoking and increase in the prevalence of *Candida albicans* infection in patients with OSMF.

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