Original Article

Association Between Smoking and Smokeless from of Tobacco With candida Species in The Oral Cavity.

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Abstract

Candida species constitute a part of the human oral commensal flora in 2 to 71 percent of healthy subjects. Several previous studies have reported that tobacco smoking, either alone or in combination with other systemic or local factors, is associated with increased oral candidal colonization or with the development of oral candidosis. It has been suggested that cigarette smoking might lead to localized epithelial alterations allowing candidal colonization. Cigarette smoke may also provide nutrition for candida albicans. Theories offer partial explanations why smokers may be more prone to candidal leukoplakia with higher potential for malignant changes than other leukoplakias. Aim of the study was to assess and compare the quantitative oral colonization of Candida species between a group of tobacco smokers and smokeless form of tobacco users

Keywords: smokers, candida, Tongue, Oral swab, Hichrom candida differential medium.

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Introduction

Candida species constitute a part of the human oral commensal flora in 2 to 71 percent of healthy subjects.[1] It is known that Candida albicans is the most commonly isolated pathogen from oral mucosa of both healthy individuals and the patients [2,3]. However, under opportunistic conditions, such as immunosuppression and tobacco chewing, these fungi become opportunistic pathogens.[4]. The term albicans means ‘whitish’ in latin, candida albicans appear as small round fungal hyphae. Candida is the most common cause for fungal infections worldwide.

It was proposed that Candida increased epithelial atypia and lead to epithelial hyperplasia and malignant changes [5, 6]. It was stated that the risks for oral Candida carriage were associated with advanced age, female gender, pregnancy, wearing of dentures, immune suppression, hypovitaminosis, iron deficiency, steroid treatment, poor oral hygiene, systemic diseases (eg. Diabetes Mellitus), and tobacco usage [7, 8, 5, 9-17]. There are about 200 candida species, many are normal commensals, it become pathogenic in conditions of alteration in epithelium of oral mucosa and in suppression of immune system.

In the literature, while some studies revealed that the rate of oral Candida carriage was higher among smokers and smokeless tobacco users compared with non-smokers [18,19]. A possible explanation for this association is the presence of nicotine and hydrocarbons such as polycyclic aromatic hydrocarbons (such as N-nitrosobenzylmethylamine), acting as nutrients for oral yeasts and intern facilitating their growth[20]. Candida growth is facilitated in poor oral hygiene conditions. Candidal growth is associated with severe neutropenia, so it can invade deeper tissues like gut.

It has been suggested that cigarette smoking might lead to localized epithelial alterations allowing candidal colonization [21]. Cigarette smoke may also provide nutrition for candida albicans [22]. This assumption has important implications as the aromatic hydrocarbons contained in cigarette smoke may also be converted, by inducible enzyme systems present in candida species, into carcinogen end products [20,23]. These theories offer partial explanations why smokers may be more prone to candidal leukoplakia with higher potential for malignant changes than other leukoplakias [24,25].

Several previous studies have reported that tobacco smoking, either alone or in combination with other systemic or local factors, is associated with increased oral candidal colonization or with the development of oral candidosis[26-28, 18] While some studies have suggested that smoking does not affect Candida carriage significantly [29-34]. Cigarette smoking seems to have a contributing effect especially on the incidence of pseudomembranous candidiasis in immunocompromised individuals [35]

Adolescents are vulnerable targets for the tobacco industry, being easily influenced by television, cinema, advertisements, and by their peers. Studies reveal that tobacco abuse is rising in adolescent age group in India, making the adolescents vulnerable to several health complications.[4] Tobacco use is a primary cause of many oral diseases and adverse oral conditions [36]. In some industrialized countries studies have shown that smoking is responsible for more than half of the periodontitis cases among adults [37]. Consumption of tobacco products remains to be an important problem of public health. According to predictions of the WHO, 22% of the people over 15 years worldwide [38].

Tobacco in its many forms is a risk factor for various systemic diseases, periodontal disease, and gingivitis.[39] Lack of awareness of the effects of tobacco use and the difficulty to discontinue the habit has led to the increased incidence of tobacco use. Tobacco habit encountered around the world is mainly in the form of tobacco smoking, tobacco chewing, and tobacco snuff use. but in India, tobacco is used in the form of bidis (34%), cigarettes (30%), chewing tobacco (19%), hookah (9%), cigars and cheroots (5%), and snuff (2%).[40]
Smoking has many negative effects on the oral cavity. Staining the teeth and restorations, decreasing the ability to smell and taste and the formation of oral lesions like smokers’ palate, smokers’ melanosis, hairy tongue, and possibly oral candidiasis, tooth decay, periodontal disease, failure of implant treatment, cancer and precancerous lesions of oral cavity are some of the effects that can be noted[41]. Hence the present study to be done to assess oral colonization of Candida species between a group of tobacco users and a comparable group of nonsmokers, and to investigate a possible correlation between oral candidal colonization and the quantity of the smoking habit.

Materials and methods
The study was conducted in Chennai among employees of Gateway Industries, Manali. Study participants are truck drivers and majority of them are tobacco users. Study subjects are selected based on inclusion and exclusion criteria. Prior to the start of the study ethical clearance was obtained from the institutional Ethics committee, Saveetha university. Informed consent was obtained from the participants.

Inclusion criteria:
Study subjects with age group between 20 – 55 years with habit of tobacco usage were included in the study. Subjects using smoking, smokeless and both form of tobacco for a period of 1 year. Subjects who is willing to participate in the study were included.

Exclusion criteria:
Participant who is on steroid and anti-fungal medication for past 3 months.
Study participant who has systemic disease such as diabetes mellitus, Hepatitis B and C infection.
Participant who is not willing to participate in the study were excluded.
Subjects wearing removable or complete dental prosthesis.

Study was conducted in the month of June 2017. N= 48. Sample size was calculated based on the study done by Azmi Mohamed- Ghaleb Darwazeh et al 2010[42]. It involves 3 groups-
Group 1: Smokers n=16
Group 2: Smokeless tobacco users. n=16
Group 3: Participant using both smoking and smokeless form of tobacco. n=16

The term “Smoker” defined a participant who smoked over 10 cigarettes a day for at least three years. Parameter of “pack years” was calculated (cigarettes per day multiplied by smoking years). The term “Nonsmoker” defined a participant who had never smoked or didn’t smoke for minimum five years.[43] The term “Healthy” defined a participant who was free of any compromised medical condition did not receive any treatment known as promoting oral candidiasis (antibiotics, steroids, high blood pressure medication, anemia due to iron deficiency, diabetes, AIDS etc.).[44].

Survey instrument:
First section includes the collection of demographic information such as name, age, gender, occupation and marital status. Second section comprise of questionnaire to assess the oral hygiene practices, about the tobacco usage, duration of usage, reason for tobacco usage and Fagestron nicotine dependence scale were used to assess the level of dependence of study participant. Third section includes isolation of candida species and samples were collected by sterile swab. Hichrom candida differential agar medium.[42] was used in the present study to assess various other candida species such as candida albicans, candida tropicalis, candida guilliermondi, candida krusei and candida glabrata
Study methodology:

**Oral swabs from tobacco users were collected in an aseptic condition**

Immediately, these swabs were inoculated on to Hichrom candida differential agar as per manufacturer instructions.

These inoculated plates were then incubated at 37 degree Celsius for 48-72 hours for colony production.

Candida species produces pigmented colonies in the given media, because it is a chromogenic medium.

The pigmented colonies make the observer easy to identify the different candida species based on the colonization as presumptive identification.

Statistical analysis: Statistical analysis to be done using SPSS software version 20. Descriptive statistics was done to assess the prevalence of various candida species among the groups. Fisher's exact test was used to determine the significant differences in the prevalence rates of oral candida carriage between the groups

Results

Figure 1 depicts the distribution of study subjects based on age group. There are about 23(48%) and 12(25%) of study subjects belong to 20 to 30 years and 31 to 40 years. About 8(17%) and 5(10%) of study population belong to the age group of 41 to 50 years and 51 to 60 years. Table 1 depicts the characteristics of study population. Mean age of the study participant were 34.5, Mean number of years of smoking was 9.08 and Mean number of cigarettes consumed daily was 5.67 respectively. Table 2 depicts the oral hygiene practices among study population. Prevalence of once brushing among smokers was 14(87.5%), smokeless form of tobacco users 13(81.3%). Prevalence of once tongue cleaning among smokers 15(93.7%), smokeless form of tobacco users 16(100%) and smoking and smokeless form of tobacco users 14( 87.5%). Table 3 depicts the candida species isolated among smokers, smokeless and both form of tobacco users. Candida albicans was found among 6(36%) of smokers, 1(6.3%) of smokeless form of tobacco users and 2(12.5%) of smoking and smokeless form of tobacco users. Candida tropicalis was found among 2(12.5%) of smokers, 1(6.3%) of smokeless form of tobacco users. Candida Krusei was found among 1(6.3%) and 4(25%) of smoking and smokeless form of tobacco users. Candida guilliermondi was found among 2(12.5%) of smokeless form of tobacco users. About 1(6.3%) of smokeless form of tobacco users were isolated with candida albicans+candida glabrata and candida albicans +candida krusei. Table 4 depicts the candida colonies among smokers, smokeless form of tobacco users, smoking and smokeless form of tobacco users. Candidal colonies count among smokers, smokeless form of tobacco users and smoking and smokeless form of tobacco users were 24, 19 and 22 candidal colonies respectively.
Figure 1: Distribution of study subjects based on age group

Table 1: Characteristics of study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>34.5±9.57</td>
<td>20-60 years</td>
</tr>
<tr>
<td>Mean number of years of smoking</td>
<td>9.08±7.75</td>
<td>3-16 years</td>
</tr>
<tr>
<td>Mean number of cigarettes consumed daily</td>
<td>5.67±3.52</td>
<td>3-10 per day</td>
</tr>
</tbody>
</table>

Table 2: Oral hygiene practices among study population

<table>
<thead>
<tr>
<th>Oral hygiene practice</th>
<th>Smokers n=16</th>
<th>Smokeless tobacco users n=16</th>
<th>Smoking and smokeless tobacco users n=16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Tooth brushing status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once brushing</td>
<td>14</td>
<td>87.5</td>
<td>13</td>
</tr>
<tr>
<td>Twice brushing</td>
<td>2</td>
<td>12.5</td>
<td>3</td>
</tr>
<tr>
<td>Tongue cleaning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once a day</td>
<td>15</td>
<td>93.7</td>
<td>16</td>
</tr>
<tr>
<td>Twice a day</td>
<td>1</td>
<td>6.3</td>
<td>0</td>
</tr>
<tr>
<td>Candida species</td>
<td>Smokers n=16</td>
<td>Smokeless form of tobacco users n=16</td>
<td>Smoking and smokeless form of tobacco users n=16</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
<td>------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>candida albicans</td>
<td>6*</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td>candida tropicalis</td>
<td>2</td>
<td>12.5</td>
<td>1</td>
</tr>
<tr>
<td>candida Krusei</td>
<td>1*</td>
<td>6.3</td>
<td>0</td>
</tr>
<tr>
<td>candida glabrata</td>
<td>1</td>
<td>6.3</td>
<td>0</td>
</tr>
<tr>
<td>Candida guilliermondi</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Candida albicans+candida glabrata</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Candida albicans+candida krucei</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Fischer exact test. F value - 7.112. p value - 1.00. p < 0.05 considered as significant

Table 3: Candida species isolated in smokers, smokeless and both form of tobacco users

<table>
<thead>
<tr>
<th>Group</th>
<th>Total no of candida colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>24 colonies</td>
</tr>
<tr>
<td>Smokeless form of tobacco users</td>
<td>19 colonies</td>
</tr>
<tr>
<td>Smoking and smokeless form of tobacco users</td>
<td>22 colonies</td>
</tr>
</tbody>
</table>

Table 4: Candida colonies count among smokers, smokeless form of tobacco users and smoking and smokeless form of tobacco users

Figure 2: Cultured plates showing various species of candidal growth
Discussion

Increasing level of education, income and occupation.[48] This is stated because irrespective of education, occupation and income, tobacco use is more prevalent among all socioeconomic groups of population.

In the present study candida species were identified in one third of the patients. In the similar study by Selma Muzurovic et al [43] showed that candida species were identified in about one third of the patients, and the most present was Candida albicans, which corresponds to other studies [49,50]. Some studies have shown that the rate of oral candidal prevalence was higher in tobacco smokers than in non-smokers. It is hypothesized that cigarette smoke enhances adhesion, growth and biofilm formation of C. albicans [11, 12].

In the current study about 36% of smokers were identified with colonies of candida albicans species followed by 12.5% of smokers were found with candida tropicalis. In the similar study done by Hamit sirri Keten et al[44] the most frequently isolated Candida species in all groups were C. albicans, followed by C. Tropicalis. C. albicans was the most commonly isolated species in this study among both smokers and non-smokers. This is consistent with others who reported that C. albicans is the most commonly isolated species from the oral cavity in health carriers and oral candidosis.[14]

Another hypothesis is that tobacco content (such as nicotine, nitrosoprolin, nitrosodiethinalamine, polycyclicaroma-treshold carbons and polonium) causes a media which facilitates the proliferation of Candida species [15]. 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. In present study about 12.5% of smokeless form of tobacco users were identified with colonies of candida guilliermondi followed by candida albicans species. In the present study about 25% of both smoking and smokeless form of tobacco users were identified with candida krusei and 12.5% with candida albicans. About 1(6.3%) of smokeless and smoking form of tobacco users were isolated with candida albicans+candida glabrata, candida albicans +candida krusei. Dissimilar results were stated in the study done by Hamit sirri Keten[44] were the most frequently isolated fungal species was candida albicans followed by candida tropicalis.

Prevalence of once brushing among smokers was 14(87.5%), smokeless form of tobacco users were 13(81.3%). Prevalence of once tongue cleaning among smokers was 15(93.7%), smokeless form of tobacco users about 16(100%) and smoking and smokeless tobacco users 14(87.5%). In a study done by Tariq Abduljabbar et al [51] prevalence of once brushing among tobacco chewers was found to be 88% and none were doing tongue cleaning. The reason for once brushing could be lack of time for people, stucked with workload and neglecting the importance of twice brushing habit.

In the present study candida colonies count among smokers was found to be 24 colonies, smokeless form of tobacco users with 19 colonies. This is similar with the study done by Talia Becker et al [52] where candida
colonies count among smokers was about 26 colonies. There are several hypotheses brought in literature regarding possible connection between smoking habits and oral Candida: mucosal changes effecting the colonization of candida [9], pro-candidal factors found in tobacco [13], the correlation between acidification of saliva (caused in part by smoking) and carriage state of candida [2].

There exists a strong relationship between the tobacco particle size and tobacco related cancer and potentially malignant disorders. Over the past decades, numerous studies have been done to analyze the effect of chemical composition of these products on oral mucosa[53]. In a study done by Naziya K.B et al among dental students revealed that the mean knowledge score among males and females was 24 and 23.9, respectively. The mean attitude score was found to be high in males (32.7) when compared to females (31.78); however, the mean practice score of females (21.78) was found to be higher than males (20.06) [54]. In a study done by Sonti Sri Harsha, the study showed that the initiation age of smoking in which, it was found that more than 50% of the population started smoking between 21-25 years of age. It was noticed that more than 50% of the smokers started smoking because of peer pressure, 33.5% due to stress and 13.5% responded that insomnia, social situation and nervousness/anxiety are factors which influenced them.[55]

Tobacco affects oral health adversely. The willingness/need to quit the habit and their level of nicotine dependence must be assessed by the dental practitioner. People like to quit the habit knowing the ill-effects of tobacco. The concept of need is essential for planning and evaluation of oral health care.[56]. After assessing the level of dependence, the modalities of treatment/intervention varies from behavioural management to pharmacological management among population. Data of treatment needs to provide curative treatment to vast and diverse population as access and affordability to the dental facilities become a constraint for majority of the population.[57]. The patient's comfort is more vital to the acceptance of any intervention.[58]. Tobacco cessation measures are undertaken only when the person accepts the intervention to quit the habit.

Health education is provided about the harmful effects of tobacco on general health and oral health. Oral health education is not only directed at reducing disease and injury to the teeth and their supporting structures rather it influences on general health and promotes a feeling of well – being. [59]. In order to assess the magnitude of the preventive task it is necessary to know the extent and severity of the disease [60]. Knowledge about ill effects of tobacco use must be imparted to tobacco users, so that they can refrain from using tobacco products. It is important to instill good oral health practices from young age to ensure long term dental health and hygiene.[61]

It was shown that poor oral hygiene (increased plaque index, index oral hygiene and dental calculus index) increased oral candida carriage significantly and that Candida species were isolated from dental plaques. We consider that poor oral hygiene in smokers and smokeless form of tobacco users may contribute to higher oral Candida carriage rates in our study. In view of the findings of the current investigation, additional studies are needed to examine the effect of tobacco smoking on oral candidal prevalence in standardized study populations.
Other variables are recommended to be taken simultaneously into consideration in these studies such as salivary flow, saliva composition, and Candida adhesion to oral epithelial cells.

Conclusion

Smoking has a significant association with prevalence of candida species in the oral cavity. Among the various candida species, candida albicans was the most prevalent. Candida colonies are found to be more in number among smokers compared to healthy subjects. Smoking cigarettes and presence of Candida species in oral cavity have adverse effects on oral hygiene. Those detrimental factors highlight the necessity of patient - doctor relation for the purpose of oral health preservation. Patients should be looking for education and visit dentists regularly. Doctor should motivate and educate patients providing professional care. Good cooperative work could lead to improvement of oral health and health in general.

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Nil

Reference


