

The Association between Smoking Habits and Candida in the Oral Cavity

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Abstract

The current study examined the effect of smoking habits on the presence of candida in the oral cavity. The study group included 50 healthy smokers (age range 20-50 years, mean 26.78 years, median 25 years and SD ± 6.9). As reference we examined a group of 50 healthy non-smokers with similar form of age and gender. This study was the first to use the Diaslide device, which was modified to provide a quantitative assessment of candidal colonies.

Of all the parameters which evaluated the influence of smoking on candida, the number of colonies in saliva was the only one that was significantly higher in smokers compared to non-smokers ($p=0.037$).

The current study had demonstrated that smoking appears to have a minor influence on candidal colony growth, compared to other known factors such as dry mouth, immunodeficiency disorders or antibiotic treatment. It is recommended that the study will be repeated with participants of different combinations of age and smoking habits, and possibly research differences between varied candida species in association with tobacco smoking in the oral cavity.

Keywords: Candida; Tobacco smoking; Diaslide

Introduction

Candida albicans is the most common fungal species isolated from the oral cavity [1] and is easily cultured from the posterior dorsum of the tongue. This dimorphic fungus may present in two phases: the blastospore phase and hyphal phase. The later can penetrate deeper into the tissue especially when the host defense mechanism is compromised [1].

A number of studies have found that smoking either alone or in combination with other factors may be an important predisposing factor for oral candidiasis, although this relationship or its pathogenic influence on oral *Candida* is far from being resolved [2]. While some studies have suggested that smoking does not affect *Candida* carriage significantly [3-8], other have reported that smoking significantly increases its prevalence [9-11]. Cigarette smoking seems to have a contributing effect especially on the incidence of pseudomembranous candidiasis in immunocompromised individuals [12].

The exact mechanism by which candidal carriage may be affected by cigarette or cigar smoke is not yet established [2]. It has been suggested that cigarette smoking might lead to localized epithelial alterations allowing candidal colonization [9]. Cigarette smoke may also provide nutrition for *Candida albicans* [13]. 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 [14,15]. These theories offer partial explanations why smokers may be more prone to candidal leukoplakia with higher potential for malignant changes than other leukoplakias [16,17].

The Diaslide device is a clinical tool for identification of urine bacterial colonies [18]. The current study was the first study that used a Diaslide device which was modified by substitution of the growing media of the

original Diaslide (MacConkey agar) to the Agar Sabouraud, a candida selective media [19], containing chloramphenicol to suppress the presence of bacteria. In the present study it was used as a clinical tool to provide a quantitative assessment of candidal colonies (Figure 1).

The main purpose of this study was to validate the association between smoking and the quantitative assessment of candida colonies from the oral cavity using a modified Diaslide as a quantitative clinical tool.

Materials and Methods

The study group consisted of 50 individuals (25 men, 25 women) healthy smokers at the ages of 20-50 years (mean 26.78). The matched control group included 50 individuals (27 men, 23 women) healthy nonsmokers, at the ages of 20-50 years (mean 25.94).

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.

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.).

Samples for candidal culture were obtained from the back of the tongue and from the saliva. In order to minimize the effect on the presence of *Candida* in the oral cavity, samples were taken at least two hours after eating, drinking and any oral hygiene procedure.

Sampling methods: the quantitative assessment of candida tool

included the modified Diaslide device. The original device referred to bacterial colonies from urine [18].

The modified device platform was Sabouraud dextrose agar which is Candida selective, containing chloramphenicol to suppress the presence of bacteria. Antibiotics, stains and some salts have been added to Sabouraud medium for bacterial inhibition and as yeast indicators [19].

The device consists of a hinged case containing two opposing agar Sabouraud media separated by a sampler with a handle at one end and two bent sampler tips at the opposite end. The tips are first dipped into the sample. The sampler is then pulled out through the casing, simultaneously inoculating both agar surfaces.

Candida albicans inoculation was primarily performed in order to introduce a reference chart characteristic of candida colonies. Instrument calibration was performed based on known concentrations of Candida (CFU/mL) in order to enable the comparison of different cultures (Figure 1). Based on these calibrations the number of colonies obtained on the diaslide could be translated into a numerical quantitative extent of candidal growth.

The following results were obtained (CFU/mL);

- 10²/ml - One Colony
- 10³/ml - two colonies
- 10⁴/ml - Twenty colonies
- 10⁵/ml - above twenty colonies

Cultures have been taken from the posterior tongue (anterior to the foramen cecum), which is the most frequent area for candidal growth (1) and from saliva (collecting 2 ml into test tubes). The devices containing the samples have been incubated for 48 hours at 37 Celsius degrees and positive candida carrier was based on one or more colony growth in culture [4].

Statistical evaluations were used to analyze the findings: chi-square test was used to evaluate the association between prevalence of candida carriage and smoking. T-test was used to evaluate the association between candidal carriage and amount and duration of smoking. Mann-Whitney test was used to evaluate the association between candidal colonies count in the study group versus the control. Spearman correlation was used to evaluate the correlation between candidal colonies count and amount and duration of smoking in the study group. p < 0.05 was required for statistical significance.

Results

The associations between smoking and Candidal carriage are presented in table 1. Although the prevalence of Candida positive cultures

was higher among smokers in saliva and/or tongue, (56% and 50%) no statistical significance was found.

The contribution of the duration of smoking and the daily amount on the prevalence of Candidal carriage was examined. The findings failed to show any significant trend in each one of those two parameters on Candida carriage.

Candidal colonies count among smokers in association with the amount and duration of smoking were examined, where 3 and 5 colonies stand concentrations of over 10³ (3 colonies) and closer to 10⁴ (5 colonies) respectively. No significant association was found between the number of saliva\ tongue colonies and smoking years or cigarettes consumption.

The associations between Candida colonies Count from study group and control group is presented in table 2. The number of colonies in saliva was the only significantly finding indicating higher prevalence of candida in 50 smokers (mean 1.04 colonies) compared to only 0.5 in the 50 non-smokers (p=0.037). When those findings are transformed to CFU/mL, the average colonies count from saliva in the study group was 100 CFU/mL compared to less than 100 CFU/mL in non-smokers.

Discussion

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 [20]. Despite these evidences, there is no uniformity in literature reports regarding the correlation between smoking and oral Candida [3-11].

The present study had tested various parameters in order to describe the association between tobacco smoking and oral candida. The effect of duration and amount of smoking on the prevalence of candida was examined. Comparison was also made between cultures from the back of the tongue to those originating from saliva. We used the diaslide device [18] modified to provide a quantitative assessment of candidal colonies. The Sabouraud dextrose agar, proposed in 1894, is still the medium most frequently employed in the primary isolation of pathogenic fungi [19] and served as medium for the modified device in the present study. The course of the current study resembles the course presented by Oliver and his partners [4], since their study also included 100 participants (37 of them were smokers), and demonstrated an association between candidal colonies count and tobacco smoking, and no significant correlation between candidal carriage and the habit of smoking [4].

group	n	Carriage - saliva samples (%)	Carriage - tongue samples (%)	Carriage - total (%)
study	50	(54%)27	(42%)21	(56%)28
control	50	(44%)22	(28%)14	(50%)25

Table 1: Candidal carriage among smokers compared with nonsmokers, i.e. associations between smoking and Candidal carriage.

group	Total number of colonies originating from saliva	Total number of colonies originating from tongue
study	52	26
control	25	16
P value	0.037	0.132

Table 2: Candidal colonies count among smokers compared with nonsmokers, i.e. associations between Candidal colonies Count from research group and control group, read according to index (CFU/ mL).

- 10² /ml - One Colony
- 10³ /ml - two colonies
- 10⁴ /ml - Twenty colonies
- 10⁵ /ml - above twenty colonies



Figure 1: The original Diaslide device was a clinical tool for identification of urine bacterial colonies, hence consisted the MacConkey agar as medium.

The Diaslide device, modified by substitution of the growing media to Agar Sabouraud, was used as a clinical tool to provide a quantitative assessment of candida colonies. Instrument calibration was performed based on known concentrations of Candida in order to enable the comparison of different cultures.

The current study, as well as the study presented by Oliver and his partners [4] might possibly indicate an association between *Candida* and tobacco smoking developing while candidal carriage exists already prior to the beginning of smoking, with tobacco only contributing to increased concentration of yeast by inducing changes in mucous membranes, releasing pro-candidal factors found in tobacco [13] and acidifying salivary pH, as favored by candidal species [20].

This study suggests that smoking demonstrates a minor influence on candidal colony growth, compared to other known factors such as dry mouth, immunodeficiency disorders or antibiotic treatment.

The diaslide device was found very convenient to operate, yet possibly limited as a quantitative measurement tool for *Candida*. The device consists of a hinged case containing two opposing agar media separated by a sampler with a handle at one end and two bent sampler tips at the opposite end. The tips of the sampler are first dipped into the sample. The sampler is then pulled out through the casing, simultaneously inoculating both agar surfaces. As a result, individual colonies can be observed even when bacterial concentrations exceed 10 CFU/mL [6]. The number of colonies on the diaslide correlates linearly with CFU per mL as determined by dilution plating [18]. Its main disadvantage is that the results are restricted to orders of magnitude. Furthermore, its narrow tip might be limiting the anticipated result while taking a sample the tongue (in former studies [4,9] higher counts were demonstrated from tongue). However, further technical modifications might improve its efficacy. For example, modifying the sampler tip to be broad and flat resembling tongue-scraper.

The average age of smokers in the study group was relatively low. A more varied age group might demonstrate higher expression of the *Candida* carriers, but old age might express diverse factors such as compromised health, prescribed medications, etc.

Another limitation of this study is a relative minority of participants who have been smoking for decades and dozens of cigarettes a day (most of the smokers in the study smoked less than ten years and less than twenty cigarettes a day) and possibly a more varied group of smokers, in terms of duration and amount of smoking, would have a different impact on number of the count of *Candida* colonies. Another limitation of this study is that colonies of *Candida* were examined without separating the different *Candida* species. Duration and amount of smoking may affect certain species more than others and investigating this aspect of the association between *Candida* and tobacco smoking might demonstrate valuable results.

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