Prevalence of oral Candida carriage and Candida species among cigarette and maras powder users

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Abstract: Objective: The aim of this study was to determine the prevalence of Candida carriage and Candida species among cigarette and Maras powder (MP) users. Material and methods: This study was conducted on 180 volunteering men in 20 cafehouses in the city of Kahramanmaras, Turkey. The sociodemographic characteristics of the participants and the behaviors of MP and cigarette usage were noted down. Culture specimens were obtained from bilateral buccal mucosa and dorsum of the tongue with a sterile cotton-tipped swap. Results: The specimens were inoculated into Sabouraud Dextrose Agar. The mean age of the participants was 40.49 ± 12.89 years (min = 18, max = 87). Fifty-eight percent of the cigarette users, 56.7% of the MP users, and 36.7% of the control group were Candida carriers. The difference of Candida carriage between cigarette and MP users and the control group was statistically significant (P = 0.018 and P = 0.029 respectively). The prevalence of Candida carriage was similar between cigarette and MP users (P = 0.854). The most frequently isolated species was Candida albicans at a rate of 30% in the cigarette users’ group, 28.3% in the MP users’ group and at a rate of 18.3% in the controls. The prevalence of Candida tropicalis carriage was found to be at a rate of 20% in cigarette and 21.7% in the MP users’ group compared to 11.7% in the nonusers. Conclusions: In the present study we found that the prevalence of oral Candida carriage was significantly higher among cigarette and MP users.

Keywords: Smoke, smokeless tobacco, candida

Introduction

Oral Candida species, especially Candida albicans, are frequently isolated from the oral cavities of healthy individuals [1]. It was reported that the prevalence of oral carriage varied between 17-75% in healthy people [2]. It is known that Candida albicans is the most commonly isolated pathogen from oral mucosa of both healthy individuals and the patients [3, 4]. 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 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 [1, 2, 5, 7-16]. 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 [17-19], other studies showed similar prevalences between these groups [20, 21]. In the study of Chattopadhyay et al., it was reported that smoking HIV-infected adults had 250% increased risk of oral Candida carriage compared to non-smoking HIV-infected adults [22]. Furthermore, it was stated that the tobacco content provided a nutritious medium to enhance proliferation of Candida species [23].

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 [24] and 27.1% in Turkey smoke [25]. In South region of Turkey, a kind of smokeless tobacco, Maras powder (MP) is used as frequent as the cigarette.
According to the studies from Turkey (Kahramanmaras city), 16.0-25.1% of the male and 1.1-1.4% of the female use MP [26, 27]. MP is prepared by powderizing leaves of the tobacco plant ‘Nicotiana rustica Linn' and mixing it (≈3 g) with various amounts of ashes (≈1 g) of the wood such as oak, walnut, or grapevine, and finally, being slightly humidified in copper boilers [28]. 1-2 g of MP is used either by wrapping in a piece of cigarette paper and smoking or placing in the inner sides of the lower lip and sucking. MP usage is remarked to be responsible for moderate dysplasia in the mucosa of the lower lips; and the duration of MP use was associated with risk of oral cancer [29].

The aim of this study was to investigate Candida carriage and species in smokers and MP users.

Material and method

Ethical considerations

In accordance with Helsinki Declaration (Seoul, 2008), the approval for the study was obtained from the Clinical Researches Ethics Committee of Kahramanmaras Sutcu Imam University. Also, the participants were informed about the study and informed consents were obtained from all of the volunteer participants.

Exclusion criteria

Exclusion criteria were smoking and using MP at the same time, alcohol intake, being on treatment with antibiotics and steroids for last 2 months, taking non-steroidal anti-inflammatory medications and antifungals for last three months, having a partial or complete dental prosthesis, and having systemic diseases such as diabetes mellitus, hepatitis B and hepatitis C infections, infection with human immuno deficiency virus and acquired immunodeficiency syndrome.

Study participants

The study was conducted on 180 volunteering men in 20 cafes in the city of Kahramanmaraş, Turkey, between August 1, 2014 and August 30, 2014. 60 individuals smoking 3 cigarettes a day for at least 1 year constituted the ‘cigarette' group, 60 individuals using MP at least 3 times a year for at least 3 years constituted the ‘Maras powder (MP) group and 60 people declaring no use of tobacco products constituted the ‘control' group.

Data collection

A questionnaire composing of questions about age, duration of cigarette use (year), the daily frequency of cigarette usage, the number of packets of cigarette consumed daily, the Fagerström test for Nicotine Dependence for smokers, duration of MP use (year), the daily frequency of MP usage, the number of packets of MP consumed daily, the duration of keeping MP in mouth (minute), the part of the mouth where MP is sucked, usage of the powder with or without cigarette paper, and habit of tooth-brushing and tongue-brushing was applied to the participants. A five-point Likert scale characterized with answers such as ‘strongly disagree, disagree, neither agree nor disagree, agree, strongly agree' was used to reveal the participants’ habits of tooth-brushing and tongue-brushing, generally practiced in questionnaires. The answers to the question about the frequency of tooth-brushing and tongue-brushing were once, twice, three times, more than three times, and none.

The fagerström test for nicotine dependence (FTND)

It is a self-assessment scale of nicotine dependency which was developed by Heatherton et al. [30] and proven in terms of validity and reliability to Turkish language by Uysal et al. [31]. The scale questioning nicotine addiction has 6 items with a total score of 0-10. The scores ≤2 correspond to very low, 3-4 low, 5 moderate, 6-7 high and ≥8 to very high nicotine dependency.

Collection of oral Candida samples and culture

Sample collection was performed after a period of 2 hours that participants did not eat or drink anything. Culture specimens were obtained from bilateral buccal mucosa and dorsum of the tongue using a sterile cotton-tipped swab. Specimens were kept in sterile tubes and inoculated into Sabouraud Dextrose Agar (SDA) at 37°C for 48 hours. After the incubation period, colonies were examined for germ tube formation in order to identify species other than C. albicans. Chromogenic agar was used as an
Oral Candida carriage

Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Smokers’ group (n = 60)</th>
<th>Maras powder users’ group (n = 60)</th>
<th>Control group (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range)</td>
<td>38.9 (26-65)</td>
<td>41.9 (18-79)</td>
<td>46.5 (18-87)</td>
</tr>
<tr>
<td>Mean duration of smoking (year) (range)</td>
<td>20.4 (1-55)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean number of cigarettes consumed daily (range)</td>
<td>15.3 (3-40)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>The FTND score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very high (percentage)</td>
<td>41.7%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>High (percentage)</td>
<td>18.3%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moderate (percentage)</td>
<td>11.7%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Low (percentage)</td>
<td>20.0%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Very low (percentage)</td>
<td>8.3%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean duration of MP usage (year) (range)</td>
<td>-</td>
<td>16.4 (3-60)</td>
<td>-</td>
</tr>
<tr>
<td>Mean frequency of MP usage (range)</td>
<td>-</td>
<td>17.3 (3-50)</td>
<td>-</td>
</tr>
<tr>
<td>Mean duration of consumption of a packet of MP (day) (range)</td>
<td>-</td>
<td>2.3 (1-15)</td>
<td>-</td>
</tr>
<tr>
<td>Mean duration of keeping the MP in the mouth (range)</td>
<td>-</td>
<td>23.4 (1-200)</td>
<td>-</td>
</tr>
<tr>
<td>Wrapping the MP in paper (percentage)</td>
<td>-</td>
<td>81.7%</td>
<td>-</td>
</tr>
<tr>
<td>Using the powder without wrapping it in a paper (percentage)</td>
<td>-</td>
<td>18.3%</td>
<td>-</td>
</tr>
<tr>
<td>Part of the mouth the MP is placed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper lip (percentage)</td>
<td>-</td>
<td>36.7%</td>
<td>-</td>
</tr>
<tr>
<td>Lower lip (percentage)</td>
<td>-</td>
<td>63.3%</td>
<td>-</td>
</tr>
<tr>
<td>Tooth brushing status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once a day (percentage)</td>
<td>13.3%</td>
<td>10.0%</td>
<td>26.7%</td>
</tr>
<tr>
<td>Twice a day (percentage)</td>
<td>5.0%</td>
<td>10.0%</td>
<td>6.7%</td>
</tr>
<tr>
<td>Three times or more daily (percentage)</td>
<td>3.3%</td>
<td>1.7%</td>
<td>0%</td>
</tr>
<tr>
<td>Sometimes (percentage)</td>
<td>55.0%</td>
<td>58.3%</td>
<td>48.3%</td>
</tr>
<tr>
<td>Never (percentage)</td>
<td>23.3%</td>
<td>20.0%</td>
<td>16.7%</td>
</tr>
<tr>
<td>Tongue brushing status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once a day (percentage)</td>
<td>5.0%</td>
<td>5.0%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Twice a day (percentage)</td>
<td>1.7%</td>
<td>0%</td>
<td>6.7%</td>
</tr>
<tr>
<td>Three times or more daily (percentage)</td>
<td>1.7%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Sometimes (percentage)</td>
<td>76.7%</td>
<td>75%</td>
<td>70.0%</td>
</tr>
<tr>
<td>Never (percentage)</td>
<td>15.0%</td>
<td>20%</td>
<td>21.6%</td>
</tr>
</tbody>
</table>
alternative media for isolation of Candida species except C. Albicans.

**Tongue lesions**

The diagnoses of tongue lesions were made according to the WHO criteria (fissured tongue, hairy tongue, coated tongue, geographic tongue, and median rhomboid glossitis) [32, 33].

**Statistical analyses**

Data were analyzed using SPSS 20.0 statistical pocket program (SPSS, Chicago, IL, USA). Mean, frequency, and standard deviation values were determined. Kolmogorov-Smirnov test was used to determine if the data were normally distributed. Mann-Whitney U-test and t-test were used to determine differences between the groups. One way ANOVA and Kruskal-Wallis tests were used in order to evaluate three groups or more. Pearson and Spearman correlation tests were used to investigate relationships between the parameters. P < 0.05 was considered statistically significant.

**Results**

**Characteristics of the participants**

The study population consisted of 60 smokers, 60 MP users and 60 volunteering male not using tobacco products (control). The mean age of the participants was 40.49 ± 12.89 (min = 18, max = 87). The mean age was 38.90 ± 8.66 in smokers group, 41.92 ± 13.89 in MP users group, and 40.67 ± 15.21 in the control group. The mean age was similar between the groups (P = 0.439). Daily number of cigarettes consumed was 15.38 ± 8.18 (min = 3, max = 40) and mean smoking duration was 20.41 ± 10.68 (min = 1, max = 55) years. The mean FTND score of smokers was 3.48 ± 2.74 (min = 0, max = 9). The duration of MP use was 16.45 ± 13.60 years (min = 3, max = 60), and duration of keeping MP in the mouth, for single use, was 23.46 ± 36.06 minutes (min = 1, max = 200). The characteristics of the participants are presented in Table 1.

**Oral hygiene status**

Thirteen of the smokers (21.7%) reported to brush their teeth at least once a day, 33 of them (55.0)% reported to brush their teeth sometimes and 14 of them (23.3%) reported that they did not brush their teeth. Thirteen of MP users (21.7%) reported to brush their teeth at least once a day, 35 of them (58.3%) reported to brush their teeth sometimes and 12 of them (20.0%) reported that they did not brush their teeth. Twenty one (35%) of non-users stated to brush their teeth every day, 29 of them (48.3%) stated to brush their teeth sometimes, and 10 (16.7%) of them stated that they never brushed their teeth. Data about tongue and teeth brushing status of the participants are presented in Table 1.

**Oral Candida carriage among participants**

The frequency of Candida carriage was found in 35 (58.3%) of cigarette group, 34 (56.7%) of MP users group and 22 (36.7%) of the control group. The frequencies of Candida carriage were significantly higher in smokers (P = 0.018) and MP users (P = 0.029) compared with non-users. It was observed that the prevalence of Candida carriage was similar between smokers and MP users groups (P = 0.854). Candida albicans was found to be the most frequently iso-

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**Table 1. The Candida species isolated in smokers’, Maras powder users’ and control groups**

<table>
<thead>
<tr>
<th></th>
<th>Smokers’ group (n = 60)</th>
<th>Maras powder users’ group (n = 60)</th>
<th>Control group (n = 60)</th>
<th>All participants (n = 180)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>18 (30)</td>
<td>17 (28.3)</td>
<td>11 (18.3)</td>
<td>46 (25.6)</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>12 (20)</td>
<td>13 (21.7)</td>
<td>7 (11.7)</td>
<td>32 (17.8)</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>3 (5)</td>
<td>3 (5.0)</td>
<td>4 (6.7)</td>
<td>10 (5.6)</td>
</tr>
<tr>
<td>Candida albicans + tropicalis</td>
<td>2 (3)</td>
<td>-</td>
<td>-</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>-</td>
<td>1 (1.7)</td>
<td>-</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. Candida species isolated</td>
<td>25 (41.7)</td>
<td>26 (43.3)</td>
<td>38 (63.3)</td>
<td>89 (49.4)</td>
</tr>
</tbody>
</table>
Oral Candida carriage

lated Candida species being detected in 18 (30%) of smokers, 17 (28.3%) of MP users, and 11 (18.3%) of the non-users. Candida tropicalis carriage was found in 12 (20%) of smokers, 13 (21.7%) of MP users and 7 (11.7%) of the non-users. Carriage status of the participant was shown in the table (Table 2).

Correlations between Candida carriage and other variables

There was not a significant correlation between Candida carriage and age of smokers (P = 0.964, r = 0.006), MP users (P = 0.290, r = -0.139) and non-users (P = 0.108, r = -0.209). Additionally, there was not significant correlation between Candida carriage and number of cigarettes consumed daily (P = 0.148, r = 0.189), duration of smoking (years) (P = 0.839, r = -0.027), and FTND scores (P = 0.124, r = 0.204). While Candida carriage did not significantly correlate with duration of MP usage (P = 0.101, r = -0.214) and duration of MP absorption in the mouth (P = 0.374, r = -0.117), a significant correlation between Candida carriage and the frequency/number of MP usage a day (P = 0.031, r = 0.279) was observed. Moreover, there was not a significant correlation between Candida carriage and daily frequency of tooth-brushing (P = 0.782, r = 0.021) and tongue brushing (P = 0.361, r = -0.068).

Clinical examination; Lesions of the tongue

None of the participants had oral lesions according to clinical examinations and there was not significant differences in terms of frequency of oral lesions among the groups.

Discussion

In the present study, we found that 58.3% of smokers, 56.7% of the MP users and 36.7% of the non-users were carriers of oral Candida. It was determined that the prevalence of Candida carriage was significantly higher among smokers (P = 0.018) and MP users (P = 0.029) compared to the controls. Additionally, it was found that Candida carriage was similar between smokers and MP users (P = 0.854). 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 [20]. In the literature, while some studies revealed a significantly higher rate of Candida carriage in the smokers compared with non-smokers [17, 18], others showed similar rates between smokers and non-smokers [20, 21]. Keten et al. reported that 54% of MP users were Candida carriers while 22% of the non-users were carriers [19]. Candida carriage among betel quid (a kind of smokeless tobacco) users and non-users showed the prevalences of Candida carriage to be 73.4% in the users and 61% in the non-users [34]. The distinctness of results of the studies may stem from differences in study populations (age, gender, race, systemic diseases), content of tobacco products and the ways of using the product, diet, and genetic characteristics.

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 [35], decrease in levels of salivary immunoglobulin A [36], and suppression in functions of polymorphonuclear leukocytes [5], thus facilitating the proliferation of Candida species. It is also hypothesized that cigarette smoke enhances adhesion, growth and biofilm formation of C albicans [11, 37]. Another hypothesis is that tobacco content (such as nicotine, nitrosoprolin, nitrosodiethelainamline, polycyclicaromatichydrocarbonsandpolonium) causes a media which facilitates the proliferation of Candida species [23]. 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 [38, 39].

The most frequently isolated Candida species in all groups were C. albicans, followed by C. tropicalis, in the present study. Consistently, it has been reported in the literature that the most frequently isolated oral Candida species was C. Albicans followed by C. tropicalis both in smokers and the normal population [20, 40]. There are studies indicating that the most frequent fungal species among smokeless tobacco users; gutka [41] and betel quid [34], was C. albicans followed by C. tropicalis. It has been reported that the fungal species most frequently isolated from the oral mucosa of MP users was C. albicans, followed by C. glabrata [19]. Our study results are consistent with the literature.
Oral Candida carriage

In this study, no significant differences were observed between Candida carriage and age, the number of cigarettes consumed daily, duration of smoking (year), the FTND score, duration of MP usage, and duration of keeping MP in the mouth. However, a significant correlation between the frequency of MP usage and Candida carriage was found. In another study on MP users, there was not a significant correlation between Candida carriage and age, frequency of MP usage, daily packages consumed, duration of MP exposure, and duration of keeping in mouth [19]. In another study on betel quid users, it was also shown that Candida carriage was not correlated with age, duration of betel quid usage, and the number of betel quid chews daily [34]. The results of the present study are consistent with the literature.

It was shown that poor oral hygiene (increased plaque index, index oral hygiene and dental calculus index) increased oral candida carriage significantly [40] and that Candida species were isolated from dental plaques [42]. We consider that poorer oral hygiene in smokers and MP users may contribute to higher oral Candida carriage rates in our study.

Conclusion

We found that the prevalence of oral Candida carriage was significantly higher in smokers and MP users compared to non-users. 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.

Disclosure of conflict of interest

None.

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