# A Comparison of the Prevalence Rate of Oral Candida Colonization between Opium Users and Cigarette Smokers in Kerman, Iran

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# Abstract

**Original Article** 

**Background:** Candidiasis is the most common opportunistic oral infection and smoking is considered as one of its well-known risk factors. However, it remains unknown whether opium users are susceptible to increased oral candida colonization. The aim of the present study is to compare the prevalence rate of oral candida colonization between opium users and cigarette smokers in Kerman, Iran.

**Methods:** This case-control study included 75 healthy male respondents divided into three groups (25 in each group): cigarette smokers, cigarette and opium users, and non-smokers as control group. The samples were obtained from oral mucosa by scraping the mucosa with a sterile cotton swab then inoculated into Sabouraud Dextrose Agar (SDA) and CHROMagar plates and also examined with the light microscope.

**Findings:** Candida was identified in 38.70% of respondents. The most frequently isolated species was Candida albicans (90.66%). The highest prevalence of candida carriage was found in cigarette smokers (52.00%). The difference of candida carriage between the two groups of cigarette smokers and cigarette plus opium users and the control group was statistically significant (P = 0.007 and P = 0.015, respectively).

**Conclusion:** In the present study, it was revealed that the prevalence of oral candida carriage was significantly higher among cigarette and opium users in comparison to the non-users.

Keywords: Candida; Oral colonization; Smoking; Opium

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# Introduction

Candida is a short term for a group of fungi that includes certain types of yeasts. Candida strains reside in the oral mucosa in healthy individuals without any specific harm and are useful in maintaining the balance of the normal oral flora. However, when the balance is disturbed, a fungal infection develops.1 Candidiasis or oral candidiasis (OC) is the most common fungal infection in humans, and the yeast form of this fungus can be isolated from the oral cavity of 54.0-71.4% of healthy individuals, referred to as 'asymptomatic carriers'.2,3 The most common species of Candida in the oral cavity is albicans; however, other species (such as glabrata, krusei, and tropicalis) are also found in immunocompromised individuals.<sup>1,2</sup>

OC occurs when local or systemic predisposing conditions pave the way for the shift of fungal proliferation from saprophytic to parasitic state. In such a case, the fungus must be considered a pathogen instead of normal flora. The prevalence of OC for the majority of these etiologies is known. Candida strains have been isolated from the oral cavities of 45.00% of infants, 50.00-60.00% of individuals wearing a removable denture, 68.85% of nursing home inmates, up to 87.00% of patients with Sjögren's syndrome (SjS), 90.00% of patients with acute leukemia undergoing chemotherapy, and 95.00% of patients with human immunodeficiency virus (HIV).1-7

The habit of smoking has been recognized as an important predisposing factor for OC because it provokes increased keratinization in oral epithelium, in addition, the smoke constituents increase fungal virulence. The results of several studies have shown that smoking provokes increments in OC carrier state.<sup>1,3,8-14</sup> However, the results of studies on the prevalence of OC in smokers are varied and, in some cases, contradictory; therefore, there are no definite statistics about the OC pattern in tobacco users.<sup>8-20</sup>

Opium use, as an addictive drug, is prevalent in Kerman Province, Iran, the largest province in the southwest of Iran. However, there is a paucity of evidence on opium. Besides, no study has evaluated the prevalence of candidiasis in opium users despite the concomitant use of opium and cigarettes. Therefore, the present study aims to compare the prevalence of OC among smokers with those using opium and smoking cigarettes.

# Methods

In this case-control study, the subjects consisted of patients referring to the School of Dentistry, Kerman University of Medical Sciences and Dental Clinics and those referring to methadone maintenance therapy (MMT) centers in Kerman. sampling The convenience method was implement to select the subjects from among those entering the centers. The patients were settled in three groups: opium and cigarette consumers, smokers, and those who did not use any. Given the similar studies, the sample size was estimated as 75 (n = 25 in each group). The inclusion criteria consisted of oral consent, an age range of 18-60 years, no history of any known systemic diseases, a history of smoking and opium use for at least one year before the study, and daily and continuous consumption of 3 cigarettes to two packs of cigarette. Due to the limited sampling, the present study was performed exclusively on males. The exclusion criteria consisted of a history of oral administration of any topical medication or systemic medication six weeks before the study, use of other forms of tobacco or alcohol other than cigarettes and opium, a history of xerostomia, patients under orthodontic treatment or wearing any type of removable prosthetic appliance, and a history of radiation therapy to the head and neck.1-3

The data were collected by preparing oral smears from the three groups. The oral examinations were performed on a chair under adequate illumination (for the group examined in MMT centers) and on a dental unit (for the other two groups) using disposable gloves and masks for the examiner and disposable intraoral examination sets. A senior dental student examined the patients' oral mucosa for any clinical forms of acute candidiasis, including pseudomembranous (rash) and erythematous, or chronic candidiasis, including erythematousand plaque-like), hyperplastic (nodular or candida-related lesions. including angular cheilitis. denture stomatitis, and median rhomboid glossitis. If any of these conditions were present, smears were prepared from the surface of the relevant lesions. Clinical forms of candidiasis were categorized according to the World Health Organization (WHO) classification.<sup>2</sup> The examiner was trained to diagnose the different forms of candidiasis, and when in doubt, he consulted

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with a specialist on oral diseases. If the patient did not have any clinical symptoms of candidiasis, the smear was taken from the mucosa of the dorsum of the tongue, vestibule, palate, and cheeks. To prepare the smear, a sterile swab was applied to

the relevant mucosal surface and the collected tissue was spread on the slide. The sample was then fixed using 96% alcohol for 20 minutes. In the next step, the samples were immediately transferred to the medical mycology laboratory for staining and culturing procedures.

Candida is a round-to-oval yeast measuring  $3-30 \ \mu\text{m}$  in diameter that grows and proliferates in a solid culture medium and its colonies can be seen macroscopically after 24-48 hours. In the laboratory and before staining and cultivation, the samples were stored at 4 °C in a refrigerator. Then, the following four phenotypic methods were used to evaluate the Candida's presence in the collected samples:<sup>7</sup>

Periodic acid-Schiff (PAS) staining, culturing on Sabouraud Dextrose Agar (SDA) medium (25 °C or ambient temperature) (Merck, Germany) culturing on CHROMagar culture medium (green-purple color change) (India) and direct examination under a light microscope. A medical mycologist performed the evaluations above without any knowledge of the patients' cigarette smoking or opium use status.1-3 The results of the two culture media were interpreted as positive and negative, the staining results as negative and veast growth, and the microscopic examination results as negative and observation of yeast.<sup>21</sup> In the laboratory, the direct examination of two smear plates was carried out, one by adding 10% potassium hydroxide (KOH) and one with gram staining, under a hood. For the SDA medium, a line was drawn on a plate containing the culture medium, and the swab was dipped into that line. The liquid was then centrifuged, and the process was completed by removing the resulting sediment from the centrifuge with a loop. The

Candida colonization was assessed using culture media and morphological criteria using light microscopy. A part of the examination of the results was carried out visually by observing the growth pattern of Candida albicans (C. albicans) on the culture medium. In the quantitative comparison, the chi-square test and Pearson's correlation coefficient (two-tailed significance) were employed to compare the Candida colony counts formed in the three groups.

The patients voluntarily participated in the present study, and the names, addresses, or telephone numbers of the patients were not questioned so that they would not worry about their participation. The study protocol was approved by the Ethics Committee of the Vice Chancellery for Research and Technology, Kerman University of Medical Sciences under the code IR.KMU.REC.1395.402.

# Results

The 75 participants of the study were in the age range of 18-60 years, with the mean ages of 34.12, 35.92, and 38.04 years in the three groups of opium + cigarette, cigarette, and control, respectively, with no significant differences (P > 0.050). From a diagnostic viewpoint, the four techniques used to determine the presence or absence of OC in the subjects were similar; in the case of strains other than C. albicans, only the CHROMagar culture medium was positive for seven samples (9.3%) (two C. tropicalis samples in cigarette and one in cigarette + opium group, four C. dubliniensis cases in the cigarette group).

Table 1 presents the positive and negative cases of C. albicans separately in the three study groups. The highest frequency was recorded in the cigarette group, with the lowest in the control group. The overall frequency of Candida in the samples was 38.7%. The Pearson's correlation coefficient (Asymp two-tailed significance) among the three groups was calculated as 0.017.

Groups	Frequency of Candida (%)	Candida absence (%)	Percentage of Candida attendance to total	Percentage of Candida absence to total	Total (%)
Control	4 (16.0)	21 (84.0)	5.3	28.0	25 (100), 33.3
Cigarette smoking	13 (52.0)	12 (48.0)	17.3	16.0	25 (100), 33.3
Cigarette + Opium	12 (48.0)	13 (52.0)	16.0	17.3	25 (100), 33.3
Total	29 (38.7)	46 (61.3)	38.7	61.3	75 (100), 100

 Table 1. Frequency (%) of Candida detection in the three groups

Table 2. Correlation between using cigarette and o	ppium with Candida colonization
in the three groups	

Pearson chi-square (P)
$0.007^{*}$
$0.015^{*}$
0.777

Table 2 presents the comparisons of this coefficient among the groups; the difference in the colonization rate between the cigarettes + opium and cigarette groups on the one hand and the control group, on the other hand, was significant (P < 0.050). However, the difference between the cigarettes + opium and cigarette groups was not significant (P > 0.050).

Figure 1 shows the differences in culture results between the samples, and the five culture media shown here consist of (from right to left) a mixture of C. albicans and C. tropicalis, C. dubliniensis, C. albicans on CHROMagar culture medium, C. albicans on SDA medium, and negative result.



**Figure 1.** Differences in culture results (from right to left) a mixture of C. albicans and C. tropicalis, C. dubliniensis, C. albicans on CHROMagar culture medium, C. albicans on Sabouraud dextrose agar (SDA) medium, and negative result

# Discussion

The most important finding of the present study significant difference in Candida was а colonization between cigarette smokers and opium plus cigarette users on the one hand and the controls on the other hand. A search in the literature showed that no study was performed with the present design in the field of oral colonization of C. albicans in opium users. However, unfortunately, statistics show that this drug use is common in some countries, including Iran. On the other hand, Mohebbi et al. claimed that reports on opium use were not accurate and reliable and were largely underestimated.<sup>22</sup>

All the participants in the present study were male due to the limitations and it was not possible to collect samples from individuals who used only opium because they smoked cigarettes, too, in almost all cases. Meysami et al. showed a significant relationship between opium consumption and the two variables of male gender and smoking.23 Shahabinejad et al. used the term opium-addicted cigarette smoker (OACS) for individuals who smoked cigarettes and used opium at the same time. However, their study focused on the effects of this habit on hematological parameters.<sup>24</sup>

The prevalence of cigarette smoking is on the increase in developing countries, according to studies. In a study, the overall prevalence of this habit in adults in one urban area in Iran was estimated as 24.2%.25 In the present study, the prevalence rates of C. albicans in total samples, cigarette smokers, cigarette plus opium group, and control groups were 38.7%, 17.3%, 16.0%, and 5.3%, respectively. The frequency of colonization in total samples was similar to those in studies by Oliver and Shillitoe<sup>13</sup> and Rasool et al.<sup>14</sup> (35.0%) and close to this rate in the study by Muzurovic et al. (29%).8 However, it was lower for the cigarette smoking group compared to the similar studies; Rasool et al.,14 Darwazeh et al.,10 Muzurovic et al.,<sup>8</sup> and Keten et al.<sup>11</sup> estimated this figure as 58.0-57.0% and even 84.0-82.5%. If these differences can be attributed to the laboratory method of evaluating the presence of Candida, it could justify the results of studies by Darwazeh et al.<sup>10</sup> and Rasool et al.<sup>14</sup> Because, in these studies, a completely different method from that of the present study was used. However, in the study by Keten et al.,<sup>11</sup> the use of SDA was exactly similar to the present study, and only the difference in sampling from the oral mucosa or the difference in the inclusion and exclusion criteria might be considered as the reason for the differences in the results. Besides, the colonization rates of C. albicans in the control group of the Muzurovic et al. and Keten et al. studies (respectively 36.7

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and 44.0%) were much higher compared to those present study (5.3%).<sup>8,10,11,14</sup> in the The consumption rates of substances such as gutka and maras powder in studies by Javed et al.<sup>12</sup> and Keten et al.<sup>11</sup> were reported to be 57.8% and 56.7%, respectively, which are much higher compared to the opium and cigarette use in the present study (16.0%). The results of the study by Keten et al.<sup>11</sup> were similar to those of the present study; they reported significant differences for C. albicans in both smokers and maras powder consumers compared to the control group. However, Darwazeh et al.<sup>10</sup> and Javed et al.<sup>12</sup> did not report a significant difference between gutka users and the controls, and between non-smoking and cigarette smoking groups. In the present study, only C. albicans colonization was compared among the three groups, and the cause-and-effect relationship between tobacco use and this common oral mycosis was not considered. However, Muzurovic et al.8 and Oliver and Shillitoe<sup>13</sup> reported that the cigarette smoke affected the oral colonization of C. albicans and its asymptomatic carrier state. Baboni et al. reported the possible mechanism and emphasized on it.16 The exact mechanism of the exacerbation of oral Candida carrier state due to tobacco use is not yet known. However, tobacco use might cause the following:

1. It might induce changes in epithelial cells that facilitate Candida colonization.

2. Cigarette smoke might contain some nutritious factors for C. albicans.

3. Cigarette smoke might increase the level of adrenaline in the blood and increase glucose serum levels, consequently, increasing salivary glucose levels, which will be beneficial to the growth of Candida.

4. Cigarette smoke might lead to oral leukocyte dysfunction and reduce the gingival fluid secretion, thus decreasing the important immunoglobulins in it, which is considered a kind of immunodeficiency, leading to candidiasis. The majority of the available data indicate an increase in OC in tobacco use in populations that are immunocompromised, especially those infected with HIV. Therefore, it is believed that one of the most likely mechanisms in this area is a reduction in cluster of differentiation four (CD4) cell counts.<sup>15,26</sup>

One of the limitations of the present study was the sampling difficulty in opium users because they did not easily report this history. Additionally, the gold standard for OC evaluation is currently the polymerase chain reaction (PCR) technique, which could not be employed due to its high cost.

In the present study, in addition to C. albicans, the two C. tropicalis and C. dubliniensis strains were also identified in the samples. The C. tropicalis strain has been reported similarly in studies by Keten et al.<sup>11</sup> and Javed et al.<sup>12</sup> However, Keten et al. reported a 20.0% prevalence of this strain in the cigarette smoking group, which is much higher compared to that in the present study.<sup>11</sup> The C. dubliniensis detected in four samples in the smoker group did not exist in similar studies. Rasool et al. reported an 8.0% incidence for C. globular strain; such finding has not been reported in other similar studies.<sup>14</sup>

Mansour Ghanaei et al. evaluated the prevalence of oral mucosal lesions in an Iranian adult population and reported a prevalence of 1.8% for Candida infections.<sup>27</sup> The high prevalence of tobacco use in the community certainly increases the prevalence of OC. However, one of the potential problems with these evaluations, as that in the present study, is the lack of a classification system for the severity of the infection. Some mild and asymptomatic cases do not require clinical follow-ups,28 whereas more severe forms require more serious treatments. Asayama et al.29 evaluated the relationship between the clinical factors and the severity of esophageal candidiasis through a criterion called Kodsi, however such criterion has not been defined for OC.

#### Conclusion

The present study suggested that cigarette smoking and opium use increased the risk of candidiasis in the oral cavity. Further studies are necessary to examine the effect of breaking these smoking habits on reducing the colonization of this fungal species in the oral cavity in order to promote the oral health status of the patients. Promoting the knowledge of smokers and opium users could motivate them to give up cigarette smoking and opium use.

# **Conflict of Interests**

The Authors have no conflict of interest.

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## **Authors' Contribution**

Idea and managing the study, writing the manuscript: NN; laboratory assessments (mycology): SAAM; collection of data (doctoral thesis): NA.

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# مقایسه میزان شیوع کلنیزاسیون کاندیدای دهانی میان مصرفکنندگان تریاک و افراد سیگاری در کرمان

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مقاله پژوهشی

# چکیدہ

**مقدمه:** کاندیدیازیس، شایعترین عفونت فرصتطلب دهانی است و مصرف سیگار، یکی از شناخته شدهترین عوامل زمینهساز بروز آن محسوب میگردد، اما اطلاعاتی دال بر این که مصرف تریاک افراد را تا چه میزان مستعد افزایش کلنیزاسیون کاندیدای دهانی مینماید، در دست نیست. هدف از انجام پژوهش حاضر، مقایسه میزان شیوع کلنیزاسیون دهانی کاندیدا در میان مصرفکنندگان تریاک و افراد سیگاری در کرمان بود.

**روشها:** این مطالعه مورد- شاهدی، بر روی ۷۵ مرد سالم در قالب سه گروه (هر گروه شامل ۲۵ نفر) افراد سیگاری، افرادی که همزمان سیگار و تریاک مصرف مینمودند و افراد غیر سیگاری (گروه شاهد) انجام گردید. نمونهها با استفاده از کشیدن سوآپ پنبه استریل بر روی مخاط دهان تهیه شد و سپس بر روی دو محیط SDA) Sabouraud Dextrose Agar) و CHROMagar کشت داده شد و در نهایت، با استفاده از میکروسکوپ نوری مورد بررسی قرار گرفت.

**یافتهها:** کاندیدا در ۳۸/۷۰ درصد از نمونهها یافت گردید. شایع ترین گونه جدا شده، کاندیدا آلبیکنس (۹۰/۶۶ درصد) بود. شایع ترین وضعیت ناقل کاندیدا در گروه افراد سیگاری مشاهده گردید (۵۲/۰۰ درصد). اختلاف معنیداری بین وضعیت ناقل کاندیدا در دو گروه (افراد سیگاری و کسانی که همزمان از سیگار و تریاک استفاده مینمودند) با گروه شاهد وجود داشت (به ترتیب ۰/۰۰۷ = P و ۱/۰۱۵ = P).

**نتیجهگیری:** بر اساس نتایج تحقیق حاضر، شیوع وضعیت ناقل کاندیدای دهانی به صورت معنیداری در افراد سیگاری و مصرفکنندگان تریاک در مقایسه با افرادی که سیگار و تریاک مصرف نمیکنند، بالاتر میباشد.

واژگان کلیدی: کاندیدا؛ کلنیزاسیون دهانی؛ سیگار کشیدن؛ تریاک

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