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ORIGINAL ARTICLE

Candida infection in oral leukoplakia: an unperceived public health problem

Ayomi Dilhari[a], Manjula M. Weerasekera[a], Anusha Siriwardhana[a], Oshanthi Maheshika[a], Chinthika Gunasekara[a], Sunil Karunathilaka[b], Ajith Nagahawatte[c] and Neluka Fernando[a]

[a]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka; [b]Oral and Maxillofacial Unit, Colombo South Teaching Hospital, Kalubowila, Dehiwala, Sri Lanka; [c]Department of Microbiology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka

ABSTRACT

Objectives: The study aimed to determine the proportion, known risk factors and etiology for *Candida* infection in leukoplakia lesions among patients with oral leukoplakia attending the Oral and Maxillofacial Clinic at a Tertiary Care Hospital in Sri Lanka.

Materials and methods: Eighty clinically suspected oral leukoplakia patients were included. Two oral swabs each, from leukoplakia patients: one swab from the lesion and the other one from the contralateral unaffected corresponding area (as a control) were collected. Direct microscopy and culture followed by colony count and phenotypic identification were performed to identify pathogenic *Candida* species.

Results: *Candida* infection was seen in 47% of patients with oral leukoplakia. *Candida albicans* (94.7%) was the most common *Candida* species followed by *Candida tropicalis* (5.3%). Majority of *Candida*-infected lesions were seen in the buccal mucosa region. Alteration of taste ($p = 0.021$), having other oral lesions ($p = 0.008$), angular cheilitis ($p = 0.024$) and periodontitis ($p = 0.041$) showed a significant association with *Candida*-associated leukoplakia. Increasing age showed a significant tendency for *Candida* infection ($p = 0.020$). Smoking ($p = 0.026$) and betel-quid chewing ($p = 0.006$) were also found to be significantly associated, although alcohol consumption alone did not show a significant association. Oral leukoplakia patients who had all three habits: alcohol consumption, smoking and betel-quid chewing had a significant association with *Candida* infection ($p = 0.004$).

Conclusions: Patients who had a combination of risk factors: smoking, betel-quid chewing and alcohol consumption were seen to have a significant association with *Candida* infection. Further betel-quid chewing alone and smoking singly was also significantly associated with *Candida* infection in oral leukoplakia.

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Introduction

Oral *Candida* is a commensal that can develop into an opportunistic pathogen. The most prevalent and pathogenic of these species is *Candida albicans*.[1] The prevalence of *Candida* species has been reported to be 15–75% in oral cavities of healthy adults.[1–3]

Oral leukoplakia is a common potentially malignant lesion of the oral mucosa and is defined as a predominantly white lesion or plaque of questionable behaviour when clinically or histopathologically, other definable diseases have been excluded.[4] In 1966, Cawson first suggested the role of *Candida* as a promoter of provoking irreversible epithelial proliferation leading to carcinoma.[5,6] Further it is also reported that *Candida* invasion is a significant risk factor for malignant transformation of oral leukoplakia.[7] Chronic *Candida* infection presenting in the form of oral leukoplakia has been reported to have increased malignant potential compared to oral leukoplakia.[6] These lesions usually present clinically as a well demarcated, rough, raised, white plaque like lesion that cannot be rubbed off.[8] It is difficult to differentiate these lesions clinically from the commensal state by microbiological detection of the *Candida* species in the oral cavity. Therefore, additional microbiological criteria are required to diagnose *Candida* infection in leukoplakia lesions correctly.

Candida infection in leukoplakia is seen mainly in adults due to increased use of tobacco and alcohol.[9,10] Other co-factors associated are reported to be use of dentures, certain medications, oral environment and immunocompromised states such as HIV, organ transplantations, chemotherapy and diabetes mellitus.[9–11] Further advanced age, smoking, dysplasia and tongue lesions were increasingly reported to be associated with *Candida* development in oral leukoplakia lesions.[12,13] In immunocompromised patients prolong exposure to *Candida* infection may result in dissemination to blood and upper gastro-intestinal tract, resulting in significant morbidity and mortality.[14]

The proportion, known risk factors and etiological agents of *Candida* infection in oral leukoplakia has not been addressed in Sri Lanka, although it remains a challenge to

CONTACT Manjula M. Weerasekera mmweera@yahoo.com, mmweera@sjp.ac.lk Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka

the clinician to predict the outcome of these oral lesions. Early detection and identifying the causative agent would contribute greatly towards better management. Hence this study is important to provide the best treatment, to implement preventive measures and to get a good understanding of oral leukoplakia.

Material and methods

The study was a cross-sectional study that comprised of 80 clinically diagnosed cases of oral leukoplakia amongst the patients who attended the Oral and Maxillofacial clinic at a Tertiary Care Hospital in Sri Lanka. The experiments were undertaken after obtaining written consent of each subject in full accordance with ethical principles. The study was independently reviewed and approved by the Ethics Review Committee of University of Sri Jayewardenepura (MLS 2014/06) and Colombo South Teaching Hospital (No 344) in Sri Lanka.

A pre-tested interviewer administered questionnaire was used to collect the data on case history (oral/dental habits, past dental/medical history clinical presentation and treatment). According to the definition (WHO collaborating Center's Workshop (2005)), patients with oral leukoplakia were defined as 'White plaques of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer'.[15] Based on the above criteria, patients were clinically diagnosed as oral leukoplakia.

Two oral swab specimens were collected for investigations. One swab from the infected site/lesion and the other swab from an unaffected contralateral corresponding site of the mouth was taken as a control. Swab specimens were transported at 4 °C within 2 h to the Microbiology laboratory for processing. Each swab specimen was suspended in sterile phosphate buffered saline, vortex mixed and centrifuged at 6000 rpm for 10 min. The sediment was diluted with 0.1 ml sterile phosphate-buffered saline. The control sample was also processed using the same method. Fifty microlitre each of diluted sediment was transferred to Sabouraud Dextrose Agar (SDA) with chloramphenicol and spreaded evenly using a sterile spreader. The inoculated plates were incubated aerobically at 26–37 °C for 48 h. Immediately after plating, the rest of the diluted sediment was used for direct microscopy to visualize the presence of yeast cells, budding yeast cells and pseudohyphae.

Candida colonization of the site was determined as described by Fanello et al. [16] A heavy *Candida* carriage was defined as having more than 50 CFU/ml on SDA from an oral swab sample. A lesion was considered as having *Candida* infection in leukoplakia if the *Candida* colony count from the lesion was more than 50 CFU/ml and its control (from an unaffected site) was negative (no *Candida* growth) or less than 50 CFU/ml. A specimen was considered negative for *Candida* infection,

- If the *Candida* colony count from both lesion and control was negative.
- If the *Candida* colony count from the lesion was negative and its control was positive.
- If the *Candida* colony count from the lesion was less than 50 CFU/ml and its control was negative.
- If the *Candida* colony count from the lesion was less than the colony count of control.

All the study participants were then classified into two groups, '*Candida*-infected oral leukoplakia' in those patients who were positive for *Candida* by culture and 'non-*Candida*-infected oral leukoplakia' in those patients with clinically diagnosed leukoplakia but negative by culture.

The types of colonies formed, the number of colonies and any confluent growth were noted. Colonies showing confluent growth were sub-cultured onto fresh SDA plates for isolation. All the different types of colonies were observed by a Grams'-stained smear and only those positive for *Candida* were processed further. Colony morphology of *Candida* (colour, size, topography and texture) was recorded. *C. albicans* was presumptively identified by the germ tube test (on serum), corn meal agar test and growth at 42 °C on SDA. Germ tube negative non-*Candida albicans* species were identified presumptively by the carbohydrate assimilation pattern and CHROMagar Candida™ medium (HiMedia). When cultured on CHROMagar Candida™ medium (HiMedia) at 37 °C for 48 h *C. albicans* gave light green colonies while *Candida tropicalis*, *Candida glabrata* and *Candida kruzei* gave, blue, cream to white and purple, fuzzy colonies respectively.[17]

The statistical analysis was carried out by using the software, Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) version 17.0. Descriptive statistics were represented as a percentage (%) value. The statistical tests for qualitative variables were carried out using the chi-square test (χ^2 test) and Fisher's exact test. All these tests were two sided. The independent sample *t*-test was used for quantitative variables. The level of significance was taken at 5% ($p < 0.05$).

Results

Out of a total of 80 patients with oral leukoplakia, 47.5% (38/80) had *Candida* infection, of whom 34 (89.5%) were males and 4 (10.5%) females. The age range of *Candida*-infected oral leukoplakia patients were between 42 and 86 years with an average age of 61.02 years at the time of diagnosis. When ethnic variation and presence of *Candida* infection in leukoplakia was considered, 35 (92%) were Sinhalese and 3 (8%) were Tamils. Majority of these patients had a primary education (86.8%), while 13.2% had higher education. It was found that patient's gender, race and level of education had no statistical significance with *Candida* infection ($p > 0.05$) (Table 1).

The average age seen in *Candida*-infected leukoplakia and non-infected leukoplakia, were 61.02 years and 55.81 years, respectively. The mean age of patients with *Candida*-infected leukoplakia was found to be significantly higher compared with that of the patients with non-*Candida*-infected leukoplakia (independent sample *t*-test, $p = 0.020$).

Table 1. Baseline characteristics of patients with oral leukoplakia ($N = 80$).

Characteristics	Non-*Candida*-infected oral leukoplakia *N* (%)	*Candida*-infected oral leukoplakia *N* (%)	*p* Value
Total	42 (52.5)	38 (47.5)	
Age (years)			
Mean (SD)	55.81 (9.89)	61.02 (12.12)	0.020
Range	32–72	42–86	
Sex			
Male	30 (71.4)	34 (89.5)	0.054
Female	12 (28.6)	04 (10.5)	
Race			
Sinhala	39 (92.9)	35 (92.1)	-
Tamil	03 (7.1)	03 (7.9)	
Educational level			
Primary education	36 (85.7)	33 (86.8)	0.884
Higher education	06 (14.3)	05 (13.2)	

SD: standard deviation, The level of significance was taken if p value <0.05.

Table 2. Association between patients' habits and *Candida* infection in oral leukoplakia ($N = 80$).

Patients' habits	Non-*Candida*-infected oral leukoplakia *N* (%)	*Candida*-infected oral leukoplakia *N* (%)	*p* Value
Smoking			
Yes	26 (61.9)	32 (84.2)	0.026*
No	16 (38.1)	6 (15.8)	
Betel-quid chewing			
Yes	25 (59.5)	33 (86.8)	0.006*
No	17 (40.5)	5 (13.2)	
Alcohol			
Yes	24 (57.1)	28 (73.7)	0.121*
No	18 (42.9)	10 (26.3)	
Smoking + betel-quid chewing + alcohol consumption			
Yes	12 (28.6)	23 (60.5)	0.004*
No	30 (71.4)	15 (39.5)	

*p Value taken from chi-square test.

Table 3. Frequency and the duration of the habits of smoking and betel-quid chewing of the patients' with *Candida* infection in oral leukoplakia.

Smoking		Betel-quid chewing	
Frequency		Frequency	
<5 times/day	43.8%	Once a day	06.1%
5–20 times/day	28.1%	Twice a day	12.1%
>20 times/day	28.1%	>2 per day	81.8%
Duration		Duration	
<5 years	03.1%	<10 years	18.2%
6–11 years	21.9%	10–20 years	18.2%
12–20 years	21.9%	>20 years	63.6%
>20 years	53.1%		

Table 4. Comparison of clinical presentation of *Candida*-infected leukoplakia and non-*Candida*-infected leukoplakia.

Characteristics	Non-*Candida*-infected oral leukoplakia (42) *N* (%)	*Candida*-infected oral leukoplakia (38) *N* (%)	*p* Value
Signs and symptoms			
Taste alteration	2 (4.8)	9 (23.7)	0.021*
Other lesions	10 (23.8)	20 (52.6)	0.008
Dry mouth	5 (11.9)	10 (26.3)	0.099
Reddish colour tongue	6 (14.3)	2 (5.3)	0.269*
Itching	4 (9.5)	7 (18.4)	0.334*
Burning sensation	27 (64.3)	28 (73.7)	0.365
Inflammation	6 (14.3)	7 (18.4)	0.617
Gum bleeding	4 (9.5)	5 (13.2)	0.729*
Complications			
Angular cheilitis	1 (2.4)	7 (18.4)	0.024*
Periodontitis	2 (4.8)	8 (21.1)	0.041*
Oral thrush	6 (14.3)	9 (23.7)	0.282
Oral cancer	14 (33.3)	10 (26.3)	0.494
Gingivitis	4 (9.5)	5 (13.2)	0.729*
Xerostomia	1 (2.4)	3 (7.9)	0.341*

*p Value taken from Fisher's exact test.

The habits of smoking and betel-quid chewing showed a significant association with *Candida* infection in leukoplakia (Table 2). However alcohol consumption alone was not significantly associated. The subjects who were having all three habits together (smoking, betel-quid chewing and alcohol consumption) were significantly associated with the development of *Candida* infection in leukoplakia lesions (Table 2).

Among the patients with *Candida* infection who reported smoking ($n = 32$) 71.9% (23/32) used cigarettes. Two out of 32, claimed to use local cigars (6.3%) while 7/32 used both local cigars and cigarettes (21.9%). Thirty-three betel-quid chewing patients were reported to have *Candida* infection in oral leukoplakia. Of them 81.8% (27/33) used betel quid which contained all ingredients including betel, tobacco, areca-nut and slacked lime. The frequency and duration of smoking and betel-quid chewing among the leukoplakia patients with *Candida* infection are described in Table 3. There was no statistically significant association with types, frequency and duration of smoking as well as the use of other condiments with betel and the frequency of betel chewing with *Candida* infection ($p > 0.05$).

Among the study group homogeneous lesions were seen among 41 cases and non-homogeneous leukoplakia lesions were seen in 39 cases. Of them 48.7% of non-homogeneous leukoplakia lesions and 46.3% of homogeneous leukoplakia lesions were colonized by *Candida*. Further, a statistically significant association between homogeneity of the lesion and *Candida* infection could not be found. The other factors such as, oral cancers, denture wearing, having a tongue lesion, oral hygiene, certain medications (steroids) and immunocompromised states such as diabetes mellitus had no statistically significant association with *Candida* infection ($p > 0.05$) in this study cohort. In this study, cohort patients with organ transplantations, patients undergoing chemotherapy and patients with HIV were not encountered.

The sites of *Candida*-infected leukoplakia lesions were in the buccal mucosa region (47.4%), the tongue (36.8%), the commissure (23.7%), gum (7.9%), lower lip (7.9%) and hard palate (2.6%). Further among the 38 *Candida*-infected leukoplakia cases, 29 (76.3%) had a single lesion while 20 (23.7%) patients had more than one lesion in their oral cavity. When signs and symptoms were considered, alteration of the taste ($p = 0.021$) and having lesions in the oral cavity ($p = 0.008$) had a significant association with *Candida* infection. The other signs and symptoms such as dry mouth, reddish colour tongue, itching, burning sensation, gum bleeding, gum abscess, inflammation and halitosis were not statistically associated with *Candida* infection ($p > 0.05$) (Table 4). Among the study population, several had angular cheilitis (10%), periodontitis (12.5%), oral thrush (19%), oral cancer (30%), gingivitis (11.3%) and xerostomia (5%). Of them *Candida* infection was seen in 18.4%, 21.1%, 23.7%, 13.2%, 26.3% and 7.9%, respectively. Further angular cheilitis ($p = 0.024$) and

periodontitis ($p = 0.041$) were significantly associated with *Candida* infection in leukoplakia (Table 4).

Forty oral leukoplakia lesions showed pseudohyphae and/or budding yeast cells on direct Grams' stain indicating virulent forms. Only 13 controls had single or occasional yeast cells. No hyphal forms were seen in these controls obtained from the unaffected sites ($p < 0.05$). Forty-one cases of leukoplakia and nineteen controls were culture positive. Out of 41 leukoplakia cases thirty-eight were determined to have *Candida* infection ($p < 0.05$). Of them thirty-six *Candida* isolates (94.7%) were presumptively identified as *C. albicans* by the germ tube test. These 36 germ tube positive isolates were identified as *C. albicans* by their ability to grow at 42 °C on SDA and chlamydospore production on corn meal and Tween 80 agar. Non-*Candida albicans* species were further identified as *C. tropicalis* (5.3%) using carbohydrate assimilation test and CHROMagar Candida™ medium (blue to purple colour colonies).

Discussion

In the present study, 47.5% oral leukoplakia patients were identified to have *Candida* infection. Several studies done in other parts of the world had reported varying level of *Candida* prevalence ranging from 15.9% to 70%.[12,13] *C. albicans* was the most common *Candida* species isolated followed by *C. tropicalis*. This has important implication to the dental community due to the potential carcinogenic ability of *C. albicans*.[18] The findings were in agreement with the published data by Abdulrahim et al. [8] and Anwar [12] where *C. albicans* had a greater association with oral leukoplakia lesions. *C. tropicalis* which was also identified in association with oral leukoplakia in this group is reported to be the most virulent among the non albicans *Candida* species. Its presence has been reported in oral leukoplakia lesions.[19]

C. albicans is known to be more virulent due to its ability to colonize, penetrate and damage the host tissues.[1,2,20] They secrete and accumulate digestive enzymes such as aspartic protease which digests oral epithelial cell surface components and allows physical movement of hyphae into or in-between epithelial cells.[21] Importantly, *C. albicans* has the ability to produce N-nitrosobenzylmethylamine (NBMA) which is considered a potent carcinogen.[18,22] Nitrosamine binds to the DNA and forms adducts with bases, phosphate residues and hydrogen binding sites.[22] This may lead to irregularities of DNA replication, point mutations and ultimately activate specific oncogenes that initiate the development of oral cancer.[22] Hence, it is very important to minimize the colonization of oral cavity by *C. albicans*.

Smoking had a significant association with *Candida* infection in oral leukoplakia. A study conducted by Anwar [12] has also shown that smoking and older age predispose to *Candida* infection. Anwer has reported the effects of nicotine in changing the normal flora to a pathogenic type,[12] as tobacco contains nicotine this may have acted as a nutrient for *Candida* species. A recent study shows that *C. albicans* cells exposed to cigarette smoke, showed transition from blastospore to the more virulent hyphal form.[23] Cessation of smoking has been reported to result in resolution of oral leukoplakia lesions.[24] The study of Wu et al. [13] also revealed that older age (>60 years) was a significant risk factor for *Candida* infection in oral leukoplakia. We observed that the average age of diagnosis of *Candida* infection in leukoplakia was higher than that reported by Arendorf et al. [9] and Wu et al. [13]

The results of this study show a significant association between betel-quid chewing and *Candida* infection in leukoplakia. de Miranda et al. [25] have noted that betel-quid chewing could suppress the normal oral flora and allow *C. albicans* to grow. Betel-quid chewing is a common habit in Asian countries, especially in Sri Lanka. Betel-quid chewing predisposes to *Candida* infection and results in oral leukoplakia. Oral cancer incidence is on the rise in the recent past in Sri Lanka. Betel-quid chewing and *Candida* infection together predisposes patients to oral leukoplakia and therefore may have contributed to the increasing incidence of oral squamous cell carcinoma in Sri Lanka. Further in this study a statistically significant association was seen when smoking, betel-quid chewing and alcohol consumption were considered together. This is an interesting finding as these three habits are common among the males in the South Asian region. The association of these three habits together has not been considered in other similar studies.

In the present study, alcohol consumption alone was not associated with *Candida* infection in leukoplakia, the combination with betel-quid chewing and smoking showed a significant association. It is known that alcohol consumption and use of tobacco are important risk factors for squamous cell carcinoma.[26] In the oral cavity, *Candida* species have ability to convert alcohol to acetaldehyde which is highly toxic, mutagenic and carcinogenic.[26] It is also known that *C. albicans* can accumulate at higher concentration of acetaldehyde than the other *Candida* species.[26,27] Hence, it is very important to minimize the *Candida* colonization in the oral cavity although a significant association of *Candida* infection and alcohol consumption was not seen in this study. Further, we were unable to establish an association between diabetes mellitus, existing oral cancers, tongue lesions, oral hygiene, use of steroids and homogeneity of the lesion with *Candida* infection in leukoplakia in this study population.

Periodontitis and angular cheilitis were found to have an association with *Candida* infection in leukoplakia in this study. Similarly MacFarlane and Helnarska [28] have also mentioned that angular cheilitis is usually associated with oral *Candida* infection. It is reported that *Candida*-infected lesions are a problem in immunocompromised individuals, those who are HIV positive or are suffering from diabetes mellitus,[29] vitamin deficiencies or chemotherapy and radiotherapy.[30] In addition, the relationships between oral *Candida* and diabetes mellitus,[29] Sjögren's syndrome [31] and combination of chronic renal failure and haemodialysis [32] have also been reported.

It has been suggested that *Candida* infection is a superimposed secondary infection in leukoplakia, but not the cause of the disease.[33] However Daftary and Mehta stated that there is a correlation between the presence of *Candida* and the incidence of epithelial atypia.[34] In the present study, it

is difficult to correlate the role of *Candida* in leukoplakia due to the absence of histopathological data which is a limitation.

In conclusion, this study revealed that *Candida* infection was seen in 47.5% of patients with oral leukoplakia. *C. albicans* was the prominent pathogen. Smoking, betel-quid chewing and alcohol consumption are significant risk factors for *Candida* infection in oral leukoplakia.

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Disclosure statement

None to declare.

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