Candida infection in oral leukoplakia: an unperceived public health problem

Ayomi Dilhari1, Manjula M. Weerasekera2, Anusha Siriwadhana3, Oshanthy Maheshika4, Chinthika Gunasekara5, Sunil Karunathilaka6, Ajith Nagahawatte7 and Neluka Fernando3

1Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka; 2Oral and Maxillofacial Unit, Colombo South Teaching Hospital, Kalubowila, Dehiwala, Sri Lanka; 3Department 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.

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 infection 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.[8] 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 prolonged 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
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 Gram’s-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 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 (86.9%), 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).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-Candida-infected oral leukoplakia</th>
<th>Candida-infected oral leukoplakia</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>42 (52.5)</td>
<td>38 (47.5)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>55.81 (9.89)</td>
<td>61.02 (12.12)</td>
<td>0.020</td>
</tr>
<tr>
<td>Range</td>
<td>32–72</td>
<td>42–86</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30 (71.4)</td>
<td>34 (89.5)</td>
<td>0.054</td>
</tr>
<tr>
<td>Female</td>
<td>12 (28.6)</td>
<td>04 (10.5)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinhala</td>
<td>39 (92.9)</td>
<td>35 (92.1)</td>
<td></td>
</tr>
<tr>
<td>Tamil</td>
<td>03 (7.1)</td>
<td>07 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary education</td>
<td>36 (85.7)</td>
<td>33 (86.8)</td>
<td>0.884</td>
</tr>
<tr>
<td>Higher education</td>
<td>06 (14.3)</td>
<td>05 (13.2)</td>
<td></td>
</tr>
</tbody>
</table>

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).

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

*p Value taken from chi-square 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 containing 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 Gram's 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.[1,12,20] 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 that smoking 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.

References