Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2019, 19:e4802
DOI: http://doi.org/10.4034/PBOCI.2019.191.86
ISSN 1519-0501

ORIGINAL ARTICLE

Oral Candidal Carriage Among Patients with Oral Potential Malignant Disorders: A Case-Control Study

Sankar Leena Sankari[1], Krishnan Mahalakshmi[2]

[1]Department of Oral Pathology and Microbiology, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Tamil Nadu, India. 0000-0002-2860-6974
[2]Department of Microbiology, Sree Balaji Dental College and Hospital, Research Lab for Oral-Systemic Health, Bharath Institute of Higher Education and Research, Tamil Nadu, India. 0000-0003-2753-9092

Author to whom correspondence should be addressed: Professor Krishnan Mahalakshmi, Department of Microbiology, Sree Balaji Dental College and Hospital, Research Lab for Oral-Systemic Health, Bharath Institute of Higher Education and Research, Chennai - 600100, Tamil Nadu, India. Phone: +91 9444184403. E-mail: kmagvenkat@gmail.com.

Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 04 March 2019 / Accepted: 26 April 2019 / Published: 03 May 2019

Abstract

Objective: To determine the prevalence of Candida species in the saliva of patients with clinically suspected oral potentially malignant disorders (OPMD) and healthy cohorts. **Material and Methods:** Unstimulated saliva was collected from patients with OPMD (n=100) and age and sex matched healthy subjects (n=170). The samples were inoculated onto Sabouraud Dextrose Agar and incubated for a week. The colonies of the isolates were enumerated using a colony counter. The isolates were identified using standard phenotypic methods. The significance of oral candidal carriage was calculated using Independent T test. Odds and Risk ratio was calculated using Pearson's Chi-square test. **Results:** Oral candida carriage was present in 51% of patients with OPMD while healthy cohorts had a prevalence of 20.6%. A good statistical significance was observed for the prevalence of oral candidal carriage for patients with OPMD in comparison to healthy cohorts (p=0.013). Significant Odds and risk ratio was observed for the prevalence of Candida species among OPMD. Majority of the isolates in both groups were *C. albicans*. Colony forming units were high among patients with OPMD. **Conclusion:** A significant association of oral candidal carriage to oral potentially malignant disorders in comparison to healthy cohorts was observed. Candidal species may be potent risk factor for transition of OPMD to oral Squamous Cell Carcinoma.

Keywords: Mouth Neoplasms; Leukoplakia, Oral; Oral Submucous Fibrosis.

Introduction

Oral potentially malignant disorders (OPMD) include all precancerous lesions and conditions that have an increased potential for malignant transformation. Etiology of potentially malignant disorders is multifactorial. Tobacco and alcohol are considered the major risk factors [1]. Candida is recognized to have significant relation with oral potentially malignant oral disorders [2]. Candida species are opportunistic pathogens, which could associate with the virulence attributes of the organism and the host factors. Oral candidal carriage frequency changes based on certain physiological changes associated with age, mucosal changes, immunity, habits of the individual and changes in the oral microbiota [3].

The ability of Candida species to persist on mucosal surfaces of healthy individuals is by itself an important factor contributing to its virulence [4]. There is an increased risk for Oral cancer in 9-40% of candidal leukoplakias compared with the 2-6% risk of the malignant transformation of leukoplakias in general [5]. Vast majority of Candida species are found colonizing the oral mucosa as normal commensals. In health, the amount of yeast is kept under control by specific and nonspecific defence mechanisms of the saliva and the oral mucosa.

The risk of Candida species in malignant transformation may be attributed to its virulence capacity. Candida has the ability to produce carcinogenic nitrosoamine compounds, like Nitrosobenzyl methylamine (NBMA) [6]. Limited data are available on the carriage of oral Candida species in the saliva of patients with OPMD in Indian Scenario. The earlier studies have been performed with less number of samples. Studies associating oral candidal carriage and OPMD being easy and simple can help identify the high-risk individuals for malignant transformation. The present study was carried to evaluate oral candidal carriage among patients with OPMD and healthy cohorts.

Material and Methods

Sample

The study population comprised of patients with clinically suspected potentially malignant oral disorders (n=100) and healthy subjects (n=170). Patients with uncontrolled diabetes& immunocompromised status, denture wearers, patients receiving steroid therapy and under long term local and systemic drug therapy were excluded. Individuals with no oral mucosal lesions and deleterious habits were included for healthy subjects.

Unstimulated whole saliva was collected by the `draining' method .The subject's head was tilted forward so that saliva moves towards the anterior region of the mouth and the pooled saliva (2 mL) was collected into a wide mouthed sterile container [7]. The sample was then immediately transported to the Microbiology lab in for isolation and identification of *Candida albicans* and non *Candida albicans* species. 10 µl of saliva sample was inoculated onto the Sabouraud dextrose agar (SDA) plate at 37^{0}C for a week. The purity was checked by Gram staining. Colony count was performed by a digital colony counter and expressed as colony forming unit (CFU)/mL of saliva.

The identification of Candida species were by phenotypic methods (CHROM agar, germ tube tests, Chlamydospore formation on cornmeal agar, sugar assimilation and fermentation tests). Prevalence, quantification and identification of the *Candida albicans* and non-*albicans* isolates were documented.

Data Analysis

The significance of oral candidal carriage was calculated using independent T test. Odds and risk ratio with 0.95 confidence interval for prevalence of Candida among OPMD was analyzed using Pearson's Chi square test. P value <0.05 was considered significant.

Ethical Aspects

The study was cleared by Institutional ethics committee, Sree Balaji Dental College and Hospital. Informed consent was obtained from all the patients willing to participate in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Results

The OPMD comprised patients with leukoplakia (48%), oral submucous fibrosis (36%), erythroplakia (4%), erosive lichen planus (3%) and erythroleukoplakia (2%). Seven patients had a combination of leukoplakia and oral submucous fibrosis (7%). The number of males /females in OPMD and health were 99/1 and 164/6, respectively. Significant difference was not observed between OPMD patients and healthy subjects with respect to age and sex (p>0.05).

Table 1 shows the prevalence of *Candida albicans* and non-*albicans* in the study population and control group.

Table 1. Prevalence of *Candida albicans* and non-*albicans* in OPMD and health subjects.

Oral Potentially Malignant Disorders	Total	Candida species			
		Presence	Absence	C. albicans	non-*albicans*
	N (%)	N (%)	N (%)	N (%)	N (%)
Leukoplakia	48 (48.0)	22 (45.8)	26 (54.2)	15 (68.2)	7 (31.8)
Oral Submucous Fibrosis (OSMF)	36 (36.0)	20 (55.6)	16 (44.4)	18 (90.0)	2 (10.0)
Leukoplakia/OSMF	7 (7.0)	2 (28.6)	5(71.4)	1 (50.0)	1 (50.0)
Erosive Lichen Planus	3 (3.0)	2 (66.7)	1 (33.3)	2 (100.0)	0 (0.0)
Erythroleukoplakia	2 (2.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)
Erythroplakia	4 (4.0)	3 (75.0)	1 (25.0)	1 (25.0)	2 (75.0)
Total	100 (100.0)	51 (51.0)	49 (49.0)	39 (76.5)	12 (23.5)
Health	170	34 (20.0)	136 (80.0)	19 (55.9)	15 (44.1)

In OPMD, the oral candidal carriage was 51%. The percentage of *Candida albicans* and non-*albicans* was 72.4 and 27.4, respectively. Oral candidal carriage among healthy cohorts was 20%. The prevalence of *Candida albicans* and non-*albicans* was 55.1% and 44.1%, respectively. A statistically

significant difference was observed between the study and control group with respect to Candida species prevalence (p=0.013). The prevalence of *C. albicans* species was significantly high compared to non-*albicans* in the study group, while significance was not observed in the control group. Abundance of Candida colony forming units (>1000cfu/ml) was observed among OPMD. Conversely healthy subjects showed colony forming units below 1000/ml. Significant Odds ratio (4.1633; 3.6433-7.1664) and Risk ratio (2.55; 1.7848-3.6433) was observed for oral candidal carriage in OPMD.

Discussion

Candida is considered an etiologic factor for OPMD because of its ability to produce carcinogenic compounds like nitrosamines (N-nitrosobenzylmethylamine - NBMA) and its ability to convert nitrite and nitrate into nitrosamines and other substances to produce acetaldehyde [6]. A prospective study has reported the association of Candida species to leukoplakia. One among 15 patients with persistent candidal infection of the lips developed carcinoma [8]. Previous authors have revealed the progression of candidal leukoplakia to oral squamous cell carcinoma (OSCC) in six of ten patients [9]. Animal studies have proved the ability of *C. albicans* in inducing epithelial hyperplasia, increased mitotic activity, epithelial dysplasia and transforming dysplastic epithelium into malignancy [10]. The fact that epithelial dysplasia improves after elimination of Candida spp. from infected tissue also supports a causal link [11].

All the patients with OPMD in our study had the habit of smoking, tobacco chewing and consumption of alcohol. Majority of them had all the three habits. A previous study showed that current smokers were more likely to carry high Candida loads nearly seven times more than past smokers or non-smokers [12].

The results of our study showed a higher oral candidal carriage compared to several earlier studies [13-17]. Previous authors have speciated Candida by germ tube test and chlamydospore formation [15]. The prevalence of *C. albicans* with potentially malignant disorders has been investigated by various authors under microbiological [17-19], cytological [17,19] and histopathological studies [6,17,18]. *C. albicans* was the predominant isolate in our study which correlated well with previous studies [14,16]. The lower prevalence of non-*albicans* did not match with an earlier study as their study did not reveal the presence non-*albicans* [15].

The prevalence of Candida species in patients with leukoplakia was slightly lower compared to previous reports [15,20]. Conversely, it was higher than a study that has reported 40% candidal-positive culture in leukoplakia [17]. A meta-analysis on fifteen studies of leukoplakia reveals 32.2% of Candidal prevalence [21]. The prevalence of Candida species in OSMF subjects was slightly lower than that observed in Sri Lankan patients (63.6%) [18]. Conversely our study had a higher prevalence than few other studies [13,15,19,22].

It is postulated that alterations in the overlying epithelium in OSMF would breach the physiological barrier offered in healthy status, creating a favourable and conducive

microenvironment that increases the colonization of Candida [22]. The association of Candida in OSMF was higher than leukoplakia, which correlates with an earlier study [15].

Oral yeast carriage in healthy patients reported so far vary significantly ranging from 2% to 70%, most likely due to variations in sample collection methods [23]. The present study reports a higher percentage of oral candidal carriage in health compared to previous studies [14,15,22,24]. Conversely, we report a low prevalence of Candida compared to Sri Lankan population (50%) [18] and Polish population (63.1%) [25].

The significant odds and risk ratio of oral Candidal carriage among OPMD suggests its potential risk. Oral Candidal carriage may be an added burden to patients with OPMD in the transition of OPMD to malignancy.

Conclusion

The present study shows significant association of oral Candidal carriage to oral potentially malignant oral disorders. Although there has been a recent surge in reporting isolation of non-*Candida albicans* species, our study had *C. albicans* as the dominating species isolated among OPMD. A considerable proportion of cases with OPMD showed higher colony-forming units compared to controls. Patients with positive candidal growth in OPMD use of antifungal therapy can be initiated to reduce the risk for malignant transformation. The exact role of Candida as a promoter of oral cancer still needs to be evaluated further.

Authors' Contributions: SLS and KM declare that they contributed to data analysis and interpretation, article writing, relevant critical review of intellectual content and approval of the final version to be published.

Financial Support: DST- FIST (Ref. No. SR/FST/College-23, 2017), Bharath Institute of Higher Education and Research.

Conflict of Interest: The authors declare no conflicts of interest.

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