Research Paper: Candida Albicans detection in pre-malignant and malignant lesions of the oral cavity

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ABSTRACT

Introduction: Nowadays, there is a remarkably increase of the interest of Candida Albicans studies mainly because of its relation with potentially malignant and malignant lesions of the oral cavity. In the present study we aim to reveal if there are any differences between these lesions or not.

Materials and Methods: Totally 72 patient consist of 23 leukoplakia, 18 oral lichen planus, 13 well differentiated squamous cell carcinoma (WSCC), 13 moderate squamous cell carcinoma (MSCC) and 5 undifferentiated squamous cell carcinoma were included in this cross-sectional analytical study. These samples were then gone under PAS staining and statistically analyze with Mann Whitney, Chi-square (p<0.05).

Results: The results showed significantly differences between dysplastic and non-dysplastic epithelium (P=0.008), but there are no statistically significant differences between various degrees of oral squamous cell carcinoma (OSCC) (P=0.26), even though there were differences.

Conclusion: The present results confirms the previous studies on the correlation between malignant lesions and the presence of fungi hyphae, but further studies are needed.

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Introduction

Cancer is one of the biggest problem and five main cause of death which entangle many people all over the world. One of the most momentous and the sixth common cancer that encompass 2-4% of malignity in developed countries is oral cancer. (1)

Squamous cell carcinoma (SCC) is the most prevalent malignant neoplasia in oral cavity which is a multifactorial disease related to numerous genetic changes and other risk factors including inadequate oral hygiene, smoking, alcohol, UV, viral infection, immune suppression, Candida albicans infection and specially some premalignant lesions such as leukoplakia and erythroplakia. (2, 3)

Infection has an important role in cancer formation and Candida is one of the most common pathogens that can cause infection in the oral cavity.

Candida is an opportunistic pathogen and one of normal flora in oral cavity and the Candida albicans is the most common and pathogenic species which is dominant in premalignant lesions such as oral leukoplakia and erythroplakia. (4, 5)

Most recent studies indicate that Candida Albicans is capable to promote cancer by various mechanisms such as production of carcinogenic byproduct, triggering of inflammation, induction of The response and molecular mimicry. (6)

Considering that there are very few studies on the prevalence of Candida albicans in different degrees of oral SCC, in this study we compared the prevalence of Candida infection in histological sections of oral SCC lesions with varying degrees and also we examined the presence of Candida in leukoplakia and lichen planus lesions with PAS coloring.

Method and material

The present descriptive cross-sectional study was undertaken by retrieving archived records and paraffin-embedded tissue blocks of previously detected cases as oral squamous cell carcinoma, Leukoplakia and Lichen planus in the department of oral and maxillofacial pathology during 2010-2016.

On the basis of Sanketh et.al study, estimating the frequency of Candida Albicans in oral squamous cell carcinoma using White fluorescent stain, considering the percentage of success in the case group with a confidence level of 95% in total, at least 27 samples of oral SCC considered for this study. (7)

And also based on Mehdipour et.al study, prevalence of candida species in erosive oral Lichen Planus, regarding the percentage of success in case group with a confidence level of 95% at least 26 samples for each group of leukoplakia and lichen planus are considered. (8)

Finally, 72 patient records were chosen to assess samples including; 23 leukoplakia, 18 oral lichen planus, 13 well differentiated squamous cell carcinoma (WSCC), 13 moderate squamous cell carcinoma (MSCC) and 5 undifferentiated squamous cell carcinoma. The samples should have intact and adequate tissue in embedded paraffin block, and the patients shouldn't be under any treatment such as radiotherapy or chemotherapy or be under antifungals medication before doing biopsy. Age, gender, presence of dysplasia, presence of Candida Albicans and the type of lesion were the components which were considered.

Two section of 5μm thickness of each case were stained using hematoxylin and eosin (H&E) and Periodic Acid-Schiff reaction (PAS). The samples were fixed and the specimens were examined for presence or absence of fungi with a light microscope (Motik, made in Spain) with magnification of ×40 by two oral pathologists.

Statistical analysis was performed using SPSS 16, Mann Whitney, Chi-square (p<0.05). Descriptive analysis was used to report Candida Albicans presence among samples.

Results

In this experimental study after collecting and arranging information, SPSS 21, Mann Whitney and Chi-square tests were used to analyze information(p<0.05). There was no statistically significant relationship between the oral lichen planus and leukoplakia sam-

ples based on Mann Whitney test. In addition, there was no significant difference among OSCC samples based on degree of differentiation using Chi-square test. (Table 1)

According to the Mann Whitney test, there was a significant difference between the two degree of differentiation, WSCC and MSCC, (P=0.016). But, despite considerable numerical difference, no statistical significant difference between WSCC and PSCC was observed probably due to the limited sample size (P=0.268). (Table1)

Table 1. Candida Albicans presence in different grades of SCC, Leukoplakia ,and Lichen planus

Candida Lesion	Candda Albcans (+)	Candida Albcans (-)	total	P-value
WSCC	4(30.8%)	9(69.2%)	13	
MSCC	8(61.5%)	5(38.5%)	13	0.260
PSCC	3(60%)	2(40%)	5	
Leukoplakia	12(52.2%)	11(47.8%)	23	
Lichen planus	9(50%)	9(50%)	18	0.891

Table 2 shows a statistically significant difference between dysplastic and non-dysplastic epithelium in Leukoplakia and Lichen planus. (p<0.05).

Table 2.Candida Albicans presence in Leukoplakia and Lichen planus with respect to dysplasia

Candida Lesion	Dysplasia	Candida Albicans (+)	Candida Albicans (-)	total	P-value
Leukoplakia	+	9(81.8%)	2(18.2%)	11	0.008
	-	3(25%)	9(75%)	12	
Lichen planus	+	5(100%)	0(0%)	5	0.011
	-	4(30.8%)	9(69.2%)	13	

According to Table3, there is no significant correlation between the presence of Candida Albicans with respect to gender using Mann Whiney test.

Table 3.Candida Albicans presence in the study samples with reference to gender

Candida Lesion	Gender	Candida Albicans (+)	Candida Albicans (-)	total	P-value
OSCC	male	7(50%)	7(50%)	14	0.873
	female	8(47.1%)	9 (52.9%)	17	
Leukoplakia	male	9(52.9%)	8(47.1%)	17	0.901
	female	3(50%)	3(50%)	6	
Lichen planus	male	2(50%)	2(50%)	4	0.999
	female	7(50%)	7(50%)	14	

According to the results in Table 4, there is no statistically significant difference of Candida Albicans presence according to the age of samples based on Mann Whitney test.

Table 4. Candida Albicans presence in the lesions of different ages

Candida Lesion	Age	Candida Albicans (+)	Candida Albicans (-)	total	P-value
OSCC	≤65	6(37.5%)	10(62.5%)	16	0.218
	>65	9(60%)	6(40%)	15	
Leukoplakia	≤59	7(58.3%)	5(41.7%)	12	0.537
	>59	5(45.5%)	6(54.5%)	11	
Lichen planus	≤43	4(40%)	6(60%)	10	0.343
	>43	5(62.5%)	3(37.5%)	8	

Discussion

The role of pathologic significance of fungal infection in premalignant and malignant lesions is still unknown. In vitro studies show that Candida can produce carcinogenic compounds, like nitrosamines which can lead to cancerous changes. The yeast cells can extend from the epithelial surface to the deeper layers representing deposition of precursors like nitrosamines to deeper layers. This shows that certain strains of Candida albicans can play a

key role in the development of dysplasia (9).

Hyphae and fungi could be detected in the smears and in the culture, but could be lost while the laboratory procedure of the biopsy specimen is handling, making a negative result in histopathologic examination (10). Moreover, when collagen fibers form a netlike appearance using subgroups, or the existing keratinized fibers in the epithelium, it makes them difficult to distinguish from the mycelium of candida. So, although it is not the most reliable way to analyze fungi hyphae, we use PAS staining to find the differences between normal and dysplastic epithelium (10).

The results of the present study showed that there were significant differences between pre-malignant and malignant lesions. The results demonstrate that the fungal infection is higher in dysplastic lesions and also in moderately and poorly differentiated SCCs. A similar result was obtained in another study, where there is an association between fungal infection and mild, moderate and severe dysplasia and hyperkeratosis. When the degree of dysplasia increased, a significant growth in PAS positivity was noted ($P = 0.008$). In the present study, 81.8% of leukoplakia lesions were positive for fungal staining demonstrated by PAS. As it was shown in other studies, Leukoplakia with candidal infection had the higher potential to have a malignant transformation than in those not infected with Candida (10). So, although the number of cases in dysplastic and premalignant lesions were low, PAS staining can be recommended in such lesions in order to manage the cases with anti-mycotics.

The present study also revealed the significant numerical differences in the SCC cases, in a way that the higher differentiation had the lower Candida Albicans hyphae so that in SCC grade I, there were less fungi than grade II and III. Malic et al. (11) found that the amount of candida fungi in epithelium was 80%, using laser microscope. Similar to our results, Dwivedi et al. (12) and Silverman et al. (13) reported the higher amount of Candida Albicans presence in oral dysplastic lesion similar to what we found (75%). Nagy et al. (14) reported 38% of fungi in

OSCC. The findings for the presence of Candida in OSCC in both studies were higher than those of our study, which could be due to the lower accuracy PAS staining comparing to other ways of fungal detection. Moreover, the number of cases in the present study are lower than that in other studies which can lead to different results.

Conclusion

In the present study, we tried to confirm the correlation between Candida Albicans and premalignant and malignant lesions by PAS staining, but further studies are needed to approve such a correlation in order to prescribe anti-mycotic treatment prior to any other therapies and also to emphasize the importance of oral hygiene maintenance.

References

1. Sangal S ,Bhargava A ,Mehra S.K ,Dakwala F.Identification of Candida albicans by using different culture medias and its association in potentially malignant and malignant lesions.Contemp Clin Dent. 2011 Sep;7(3): 188-93.https://doi.org/10.4103/0976-237X.86454

2. Ramireza-Garcia A ,Rementeria A ,Manuel Aguirre-urizar J ,Dolores Moragues M ,Antoran A ,Pellon A ,Abad-Diaz-de-cerio A,Luis Hernando F.Candida albicans and Cancer:Can this yeast induce cancer development or progression?.Crit Riv Microbiol.2016;42(2):181-93.

3. Mun M ,Yap T , Alnuaimi A ,Adams G ,McCullough M. Oral candidal carriage in asymptomatic patients. Aust Dent J. 2016; 61: 190-5.https://doi.org/10.1111/adj.12335

4. Berkovits C ,Tóth A ,Szenzenstein J ,Deák T ,Urbán E ,Gácser A ,Nagy K. Analysis of oral yeast microflora in patients with oral squamous cell carcinoma. SpringerPlus .2016; 5(1):1257.https://doi.org/10.1186/s40064-016-2926-6

5. Rodriguez MJ ,Schneider J ,Moragues MD ,Martinez-Conde R ,Ponto J ,Aguirre J. Cross-reactivity between Candida albicans and Oral Squamous Cell Carcinoma Revealed by Monoclonal Antibody C7. Anticancer Res. 2007; 27: 3639-44 .

6. Alnuaimi AD, Wiesenfeld D, O'Brien-Simpson NM, Reynolds EC, McCullough MJ. Oral Candida colonization in oral cancer patients and its relationship with traditional risk factors of oral cancer: a matched case-control study. Oral Oncol 2015;51:139-45.https://doi.org/10.1016/j.oraloncology.2014.11.008

7. D. S. Sanketh, Shankargouda Patil, Roopa S.

Rao. Estimating the frequency of Candida in oral squamous cell carcinoma using Calcofluor White fluorescent stain. J Investig Clin Dent. 2016;7(3):304-7.https://doi.org/10.1111/jicd.12161

8.   .Mehdipour M, Taghavi Zenouz A, Hekmatfar S, Adibpour M, Bahramian A, Khorshidi R. Prevalence of Candida species in erosive oral lichen planus. J Dent Res Dent Clin Dent Prospects. 2010;4(1):14-6.

9.   S. Leena Sankari, K. Gayathri, N. Balachander, and L. Malathi. Candida in potentially malignant oral disorders. J Pharm Bioallied Sci. 2015; 7(Suppl 1): S162-S164.https://doi.org/10.4103/0975-7406.155886

10.   Jahanshahi Gh, Shirani S. Detection of candida albicans in oral squamous cell carcinoma by fluorescence staining technique. Dent Res J. 2015;12(2):115-20

11.   Malic S, Hill KE, Ralphs JR, Hayes A, Thomas DW, Potts AJ, et al. Characterization of Candida albicans infection of an in vitro oral epithelial model using confocal laser scanning microscopy. Oral Microbiol Immunol   2007;22:188-94.https://doi.org/10.1111/j.1399-302X.2007.00344.x

12.   Dwivedi PP, Mallya S, Dongari-Bagtzoglou A. A novel immunocompetent murine model for Candida albicans- promoted oral epithelial dysplasia. Med Mycol. 2009;47:157-67.https://doi.org/10.1080/13693780802165797

13.   Silverman S Jr, Luangjarmekorn L, Greenspan D. Occurrence of oral Candida in irradiated head and neck cancer patients. J Oral Med 1984;39:194-6.

14.   Nagy KN, Sonkodi I, Szöke I, Nagy E, Newman HN. The microflora associated with human oral carcinomas. Oral Oncol. 1998;34:304-8.https://doi.org/10.1016/S1368-8375(98)80012-2