

Association of Candida in Different Stages of Oral Leukoplakia

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ABSTRACT

Context: Leukoplakia represents the most common premalignant oral mucosal lesion. *Candida albicans* is frequently found in histologic sections of leukoplakia. Some *Candida* strains may have the potential to promote the development of oral cancer.

Aim: The present study was undertaken to assess the role of *Candida* infection in different stages of leukoplakia and in different grades of dysplasia through cytological methods.

Materials and methods: Thirty subjects with the diagnosis of leukoplakia were taken for the study. Another thirty subjects, who were age and sex matched with that of experimental subjects, were selected as control subjects. Based on the clinical examination and histopathological examination, required data were collected.

Statistical analysis used: Mean, Chi-square test, ANOVA and Student-Newman-Keuls test were used for statistical analysis.

Results: Of the 30 subjects with leukoplakia, 11 (37%) subjects were positive for *Candida* in the lesion and 19 (63%) subjects had no *Candida* in the lesion. Among the experimental subjects, 2 (14%) subjects with stage 1 lesion, 3 (43%) subjects with stage 2 lesion and 6 (67%) subjects with stage 4 lesion showed evidence of *Candida*. Of the 11 subjects who showed evidence of *Candida*, 2 (18%) subjects had *Candida* with no dysplasia, 3 (27%) subjects had *Candida* with mild dysplasia and 6 (55%) subjects had *Candida* with moderate dysplasia. Of the 19 subjects who have not shown evidence of *Candida*, 10 (53%) subjects were with no dysplasia, 6 (32%) subjects were with mild dysplasia and 3 (15%) subjects were with moderate dysplasia.

Conclusions: Based on the present study, it could be concluded that as the stage of the lesion increases, the presence of *Candida* in the lesion also increases. In addition to this, we were able to show that the presence of *Candida* was more common in lesions with moderate dysplasia than in lesions with mild dysplasia or no dysplasia.

Keywords: Leukoplakia, Presence of *Candida*, Grades of dysplasia, Leukoplakia stages.

INTRODUCTION

Leukoplakia is a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion.³ Among the different types of leukoplakia, the terms “candidal leukoplakia” and “hyperplastic candidiasis” have been used to describe lesions with *Candida* found in histologic sections of leukoplakia, and hyperplastic candidiasis is often cited as an oral precancerous lesion.¹

Candida albicans is frequently found in histologic sections of leukoplakia and is consistently (60% of cases) identified in nodular leukoplakias but rarely (3%) in homogeneous leukoplakias¹. Experimentally, some strains of *Candida albicans* have produced hyperkeratotic lesions of the dorsal rat tongue without any other contributing factor. In a few studies, certain strains have been shown to produce nitrosamines, chemicals that have been implicated in carcinogenesis. Some candidal strains may have the potential to promote the development of oral cancer; to date, however, the evidence suggest that such a role is largely circumstantial.

Russell and Jones (1975) and Jolan Banoczy (1982)² had shown in their study that experimental *Candida* infection in rats could produce whitish lesions with marked epithelial hyperplasia and epithelial atypia. Holmstrup and Bessermann (1983) and Jolan Banoczy (1982)² have shown that, upon treatment, non-homogeneous *Candida* infected leukoplakias convert into a homogeneous lesion, and some lesions even regressed. Krogh

et al (1987) and Jesper Reibel (2003)³ proposed that the *Candida* types isolated from nonhomogeneous leukoplakias seem to be of the more rare *C. albicans* types, some of which have a high nitroization potential suggesting endogenous production of carcinogenic nitrosamines. AW Barrett, VJ Kingsmill, PM Speight (1998)⁴ in their study to determine the frequency of fungal infection in biopsies of oral mucosal lesions inferred that there was a significant positive association of fungal infection with moderate and severe epithelial dysplasia.

M McCullougha, M Jabefl, AW Barrettb, L Baina, PM Speight and SR Portera (2002)⁵, in their investigation, assessed the presence and level of colonization of oral yeast in patients undergoing an incisional oral mucosal biopsy in order to assess whether the amount of oral yeast present correlated with the presence and degree of oral epithelial dysplastic or neoplastic change. Some of the patients (44.6%) had a histopathological diagnosis of either oral epithelial dysplasia or oral squamous cell carcinoma and the frequency of oral yeast carriage was significantly greater in these patients than those without histopathologically detected dysplastic or neoplastic oral lesions.⁶ The degree of epithelial dysplasias present in these patients also correlated with higher amounts of yeast in the oral cavity. They concluded that there was an interaction between oral carriage of yeast and oral epithelial dysplasia, however, it remains unclear how yeast infection influences the development and progression of dysplasia. Nada, Marija, Dejan, Ivana (2004)⁷, in their study, concluded that although the

significance of fungal infection was sometimes underrated, its clear association with a moderate to severe epithelial dysplasia has been established and the dysplastic lesions infected with fungi had a three times greater chance to aggravate their degree. Higher incidence of fungal infection was found with potentially malignant oral mucosal lesions, as compared with those without malignant potential.

The present study was undertaken to assess the role of Candida infection in different stages of leukoplakia and in different grades of dysplasia through cytological methods.

MATERIALS AND METHODS

Experimental procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki declaration of 1975, as revised in 2000.

Permission from the ethical committee of the dental hospital was obtained before the starting of the study for interpretation and examining subjects, for drawing 3 ml of blood and also for performing biopsy as and when needed. Also, an informed consent was obtained from the subjects forming the study sample, to participate in the study and to undergo clinical investigation and biopsy procedure, if deemed necessary, in the course of the study.

EXPERIMENTAL SUBJECTS

Inclusion Criteria

Routine intraoral examination was carried out on all subjects reporting to Ragas Dental College and Hospital, Chennai, and during soft tissue examination, subjects with well-defined white patch, localized or extensive, that is slightly elevated and that has a fissured, wrinkled, or corrugated surface or a mixed red-and-white lesion in which keratotic white nodules or patches are distributed over an atrophic erythematous background or presence of thick white lesions with papillary surfaces in the oral cavity which is consistent with the diagnosis of leukoplakia was taken for the study.

Control Subjects

Thirty subjects, who were age and sex matched with that of experimental subjects, were selected. They were divided into two groups based on the following criteria: Group 1 consisted of subjects who had no intraoral mucosal lesions and group 2 consisted of subjects who had intraoral mucosal lesions.

Procedure

Information on a detailed history of the patient was collected along with a thorough clinical examination and the findings were recorded as per the proforma.

Clinical Examination

The patient was examined intraorally under incandescent light illumination using mouth mirror and probe for white patch, localized or extensive, that is slightly elevated and that has a fissured, wrinkled, or corrugated surface or a mixed red-and-white lesion in which keratotic white nodules or patches were distributed over an atrophic erythematous background or presence of thick white lesions with papillary surfaces in the oral cavity and any concomitant lesion if present within the oral cavity was recorded and photographed.

Histopathological Examination

Under local anesthesia, an incisional biopsy was taken in relation to the lesion which included an area of normal adjacent mucosa

and sent for histopathologic examination to department of oral pathology. The slides are stained with H and E stain to evaluate the presence of dysplastic changes, if any, and PAS to identify the presence of candidal hyphae (Fig. 1).

Statistical Methods used in the Study

- i. Mean
- ii. Chi-square test
- iii. ANOVA
- iv. Student-Newman-Keuls test

RESULTS

As per the research methodology described in the above paragraphs, samples were collected and analyzed. Salient results obtained in the study are presented here:

Of the 30 subjects in group I, 11 (37%) subjects were positive for Candida in the lesion and 19 (63%) subjects had no Candida in the lesion (Fig. 2)

Of the 30 subjects in study group I, 2 (14%) subjects with stage 1 lesion, 3 (42%) subjects with stage 2 lesion, 6 (66%) subjects with stage 4 lesion showed evidence of Candida. 12 (86%) subjects with stage 1 lesion, 4 (58%) subjects with stage 2 lesion, 3 (34%) subjects with stage 4 lesion had no evidence of Candida (Fig. 3)

Of the 11 subjects who showed evidence of Candida, 2 (18%) subjects had Candida with no dysplasia, 3 (27%) subjects had Candida with mild dysplasia and 6 (55%) subjects had Candida with moderate dysplasia. Of the 19 subjects who have shown no evidence of Candida, 10 (53%) subjects were with no dysplasia, 6 (32%) subjects were with mild dysplasia and 3 (15%) subjects were with moderate dysplasia. The presence of Candida between different grades of dysplasia was not statistically significant. (p value = 0.062) (Fig. 4)

DISCUSSION

In the present study, Candida was present in 37% of the subjects with leukoplakia and 63% of subjects had no evidence of Candida. These findings were in consistent with the results obtained by McCulloch et al (2002)⁵ who have described that a spectrum of different oral mucosal lesions were associated with Candida, including chronic hyperplastic candidiasis, also known as candidal

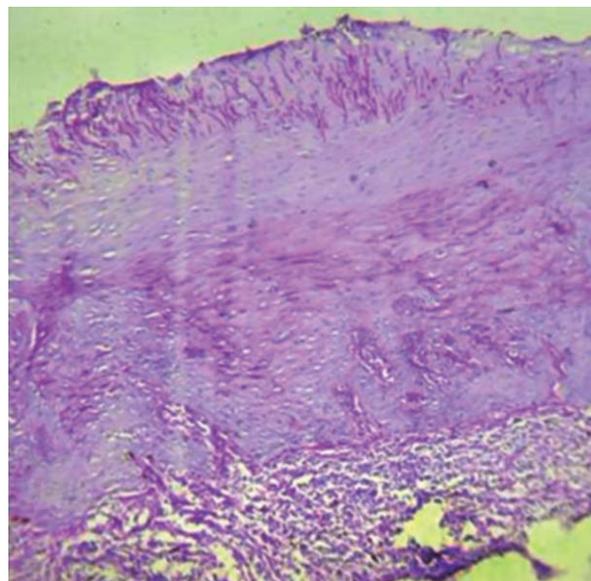


Fig. 1: PAS stain for Candida

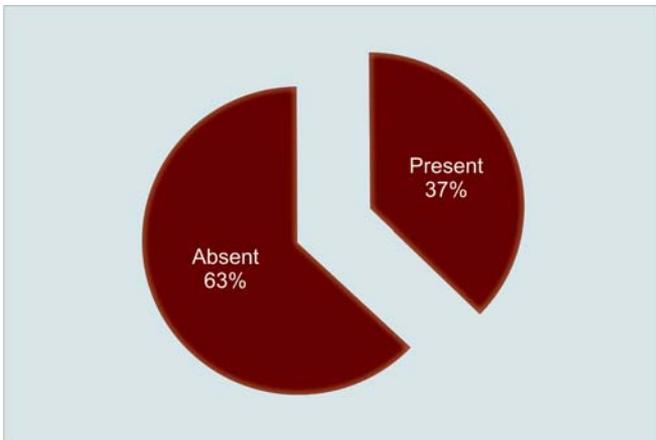


Fig. 2: Distribution of subjects with leukoplakia according to presence of Candida

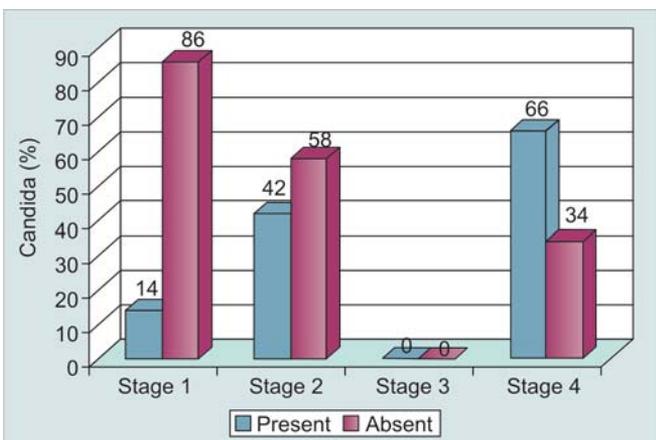


Fig. 3: Distribution of Candida in different stages of leukoplakia

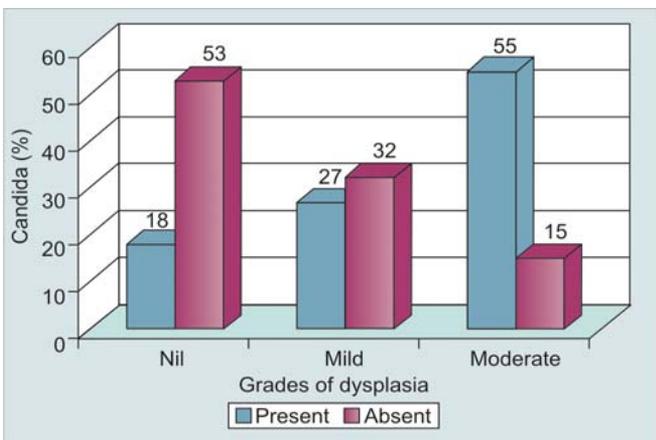


Fig. 4: Distribution of Candida in different grades of dysplasia

leukoplakia. They also suggested that this form of oral candidiasis carries a significant risk of malignant transformation.

In addition to this, in the present study, it was recorded that Candida was present in 14% of stage 1 lesion, 42% of stage 2 lesion and 60% of stage 4 lesion. 86% of stage 1 lesion, 58% of stage 2 lesion and 34% of stage 4 lesion had no evidence of Candida. Thus, the correlation between the presence of Candida

with stage of lesion was significant with p-value of 0.011. No such detailed analysis has been reported earlier.

In the present study, 55% of the lesions with moderate dysplasia, 27% of the lesions with mild dysplasia and 18% of the lesions with no dysplasia showed evidence of Candida, whereas 15% of the lesions with moderate dysplasia, 32% of the lesions with mild dysplasia and 53% of the lesions with no dysplasia showed no evidence of Candida. These findings were in accordance with the study undertaken by AW Barrett et al (1998)⁴ to determine the frequency of fungal infection in biopsies of oral mucosal lesions. They recorded 6.2% of lesions with mild epithelial dysplasia and 18% of lesions with moderate epithelial dysplasia showing the evidence of Candida. The association of moderate epithelial dysplasia with candida infection was significant with a p-value of < 0.01. In their follow-up study, they found that 21.9% dysplasias which were infected with fungi worsened in severity, and only 7.6% dysplasias which were not infected in first and subsequent biopsies showed histological evidence of progressive dysplastic changes. They concluded that there was significant association of fungal infection with moderate and severe epithelial dysplasia and dysplastic lesions infected with fungus were almost three times more likely to worsen in histologic severity. M McCullough et al (2002) described in their study that the predominant species that has been isolated from leukoplakia to be *Candida albicans*, and strain differences have been identified in leukoplakic lesions compared with normal mucosa. They have also observed that *C. albicans* with very high potential to nitrosylate N-benzylmethylamine were more likely to be isolated from advanced, potentially malignant, oral mucosal lesions.

CONCLUSIONS

Based on the present study, it could be concluded that the correlation between the presence of Candida with the stage of lesion was significant as Candida was present in 14% of stage 1 lesions, 42% of stage 2 lesions and 66% of stage 4 lesions. In addition to this, 55% of the lesions with moderate dysplasia, 27% of the lesions with mild dysplasia and 18% of the lesions with no dysplasia showed evidence of Candida. In addition to this, we were able to show that the presence of Candida was more common in lesions with moderate dysplasia than in lesions with mild dysplasia or no dysplasia. Whether Candida plays a role in the progression of dysplasia or merely affects the altered tissue is yet to be determined.

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