

Comparison of Clinical and Histopathological Behavior of Oral Leukoplakia Before and After Treatment with 1% Topical Clotrimazole - An Observational Study

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ABSTRACT

Background: The Etiological role of *Candida albicans* in malignant transformation of oral leukoplakia is much debated in the recent times. **Aim and objectives:** To compare the clinical and histopathological behavior of oral leukoplakia before and after antifungal therapy with 1% topical clotrimazole. To assess the candida colony count in saliva and correlation between salivary candidal colonies count in patients with oral leukoplakia before and after treatment with antifungal 1% topical clotrimazole therapy. **Methods:** Clinical behavior of 50 cases of oral leukoplakia was assessed and histopathological confirmed as not any other lesion. Unstimulated whole saliva sample collection for estimating candida colony count and exfoliative cytology of the lesion were performed with cytobrush to evaluate candidal hyphae and other histopathological changes using PAS and Hematoxylin-Eosin stains. Subjects were prescribed 1% topical clotrimazole thrice daily for two weeks and were recalled after two weeks and the same procedures were repeated sequentially. Paired t test was used for Statistical analysis setting the p value at <0.001. **Results:** Statistically significant difference was observed in the clinical staging, histopathological behavior and the salivary candida colony counts before and

after treatment with 1% topical clotrimazole. **Conclusion:** Significant influence of Candida in the Clinico-pathological behavior of leukoplakia merits treatment with topical antifungals like 1%clotrimazole.

Key words: Antifungal therapy, candida colony count, clotrimazole, oral leukoplakia

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INTRODUCTION

Oral Leukoplakia (OL) is the most common Potentially Malignant Disorder of the oral mucosa with a reported malignant transformation rate of 1.36%.^[1,2] Candida invasion has been suggested to be a significant risk factor for malignant transformation of OL.^[3] Literature search showed that candidal hyphae constitute 7-50% of all Leukoplakia.^[4,5] Though the role of candida on epithelial dysplasia is not fully understood, antifungal therapy is recommended to be a routine treatment for OL.^[6] Due to paucity of direct studies our research was carried out with an aim to correlate the clinical staging and histopathological behavior of leukoplakia with salivary candida colony count before and after treatment with topical antifungal therapy.

MATERIALS AND METHODS

The observational study was conducted at a Dental hospital after obtaining clearance from Institutional Review Board and Ethical Committee. Fifty subjects with clinical diagnosis of OL were included in the study after obtaining informed consent. Patients with chronic systemic illness, oral cancer, corticosteroid therapy, immunosuppressive drugs, allergic to clotrimazole, pregnancy, primary and acquired immunodeficiency were excluded from the study.

Cases were selected based on OL (OLEP) staging system proposed by Van der Waal and Axéll *et al.* [Table 1] and the information was entered in a specially designed proforma. Incisional biopsy was then performed for histopathological confirmation of the lesion. Following clinico pathologic correlation, subjects were asked to pool saliva in their mouth and the unstimulated whole saliva was collected in a sterile container. The samples were inoculated in Sabouraud's dextrose agar media at 37°C for 48 hours. Candida Colony Forming Units (CFU) was counted using the colony counter by the microbiologists at the department of Microbiology who were completely blind about the clinical staging of the lesion.

Exfoliative cytology of the lesion using cytobrush was performed to identify the presence of Candidal hyphae and other histopathological changes using PAS and Hematoxylin-Eosin (HE) stains respectively. Sitheequ suggested that histologically, Candida invasion was best done by periodic acid-Schiff (PAS) reagent staining.^[7] Subjects were counseled for habit cessation and advised to apply topical 1% clotrimazole on the lesion thrice daily for two weeks.

Method of application was explained and medication compliance charts printed in the local language with easily understandable tabular columns were given to the subjects to assess adherence to the regimen. The subjects were recalled after two weeks and assessed for clinical staging based on size, excluding the pathological aspects as per Van der Waal's Modified staging system [c-OLEP- Table 2] and saliva sample was collected to estimate the Candida colony count. Exfoliative cytology of the lesion was repeated using cytobrush to identify Candidal hyphae and other histopathological changes using PAS and Hematoxylin-Eosin (HE) stains respectively. The obtained data was tabulated and subjected to statistical analysis using SPSS software version 20. Paired t test was used to compare the clinical staging and salivary colony counts before and after treatment. Chi square test was done to compare the presence of candida hyphae, setting p value at <0.001.

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Table 1: Classification and Staging system for Oral Leukoplakia (OLEP)-Vander Wall (2002)

L (size)	
L ₁	Size of single or multiple leukoplakia together ≤ 2 cm
L3	Size of single or multiple leukoplakia together ≥ 4 cm
Lx	Size not specified
P (pathology) P0	No epithelial dysplasia (includes "no or perhaps mild epithelial dysplasia")
P ₁	Distinct epithelial dysplasia (includes "mild to moderate" and "moderate to possibly severe" epithelial dysplasia)
Px	Absence or presence of epithelial dysplasia not specified in the pathology report
OLEP staging system:	
Stage I	L1P0
Stage II	L2P0
Stage III	L3P0 or L1L2P1
Stage IV	L3P1

Table 2: Van der Waal-a modified classification and staging system for oral leukoplakia: c-OLEP system

STAGING	GROUPING
Stage I	L1C1
Stage II	L2C1
Stage III	L3C1 or L1L2C2
Stage IV	L3C2.

C1-Homogenous

C2- Non homogenous

RESULTS

There was no attrition of subjects and medication compliance was found adequate with all the subjects following the instructions. The age distribution ranged from 20-70 years, with a mean age of 50.14 ± 10.76 years. Peak occurrence of OL was observed between ages 51-60 years with a male predilection *i.e.* 94%, compared to 6% of females. The entire study group had deleterious oral habits. Of which 32 patients (64%) had smoking habits, 18 cases (36%) had smokeless tobacco (chewing) habit, and 28 cases (56%) had the habit of using both smoking and smokeless tobacco. Site predilection was highest with buccal mucosa constituting 90% of the cases followed by labial mucosa and vestibular region 4% each and 2% of cases in the retro molar region.

Among the study group (n 50) 43 cases (86%) quit their habits completely in the post-operative visit and 7 cases could not stop the habit completely. Histopathological grading showed 40% of leukoplakia cases with mild dysplasia, 2% cases with moderate dysplasia and 58% without any dysplastic features. Among the types of Leukoplakia, 39 cases (78%) were homogenous and 11 cases (22%) were non homogenous. Out of 39 homogenous leukoplakia patients candidal hyphae were observed in 11 cases and in 11 cases of non-homogenous patients showed candidal hyphae in 10 cases. There was complete elimination of candidal hyphae in cytospin smear in all the 50 cases following treatment. Chi square test showed a statistically significant p value of <0.00.

Salivary Candidal colony count ranged between 0-8000 CFU/ml, out of which only 1 case (2%) was in the normal limits of 0-600 CFU/ml. The rest of the 49 cases (98%) had a count of 800-8000 CFU/ml before treatment. Following treatment, 35 cases (70%) showed Candidal count at normal limits of 0-600 CFU/ml and 17 cases (30%) showed reduction of Candidal counts to 0-2000 CFU/ml. Paired t test showed statistically

significant reduction in CFU with a p value of <0.001. The pre and post treatment CFU counts are as shown in Table 3.

DISCUSSION

OL is regarded as a premalignant or, synonymously a potentially malignant or precancerous lesion. *Candida albicans* is the most prevalent fungus associated with about 7-50% of oral leukoplakias.^[8,9] Candida invasion has been suggested to be a significant risk factor for malignant transformation of oral leukoplakia.^[10]

By definition, there is no clear correlation between clinical and pathologic diagnosis of Leukoplakia as clinically similar lesions may be histologically different. Therefore, biopsy should be performed in all cases to determine potential malignancy.^[11] A staging system for OL (OLEP) combining the clinical aspects and histopathological findings has been proposed by Van der Waal. If the staging system should be used for studies in which no biopsies are available (in our study, follow up biopsy was not recommended due to ethical issues) one could use a symbol for the clinical (C) subdivision of OLEP staging (C1=homogeneous; C2=non-homogeneous) instead of the one for pathology. With reference to the original classification and staging system (Van der Waal), this would result in a c-OLEP system, recognizing four stages.

Among the types of Leukoplakia, the homogenous type is more prevalent than the non-homogenous type [Table 2].^[11] Our study showed similar findings constituting of 78% of cases of homogenous leukoplakia compared to 22% of non-homogenous leukoplakia.

Among the 50 cases included as per the defined inclusion criteria, the present study showed male predilection of 94% (Male: Female ratio of 15.2:1) with peak occurrence observed between the age group of 51-60 years which is consistent with the study done by Banoczy.^[3]

Jepsen and Winther found a strong association between candidal invasion and speckled leukoplakia which was later confirmed in many studies.^[5] Vander waal recommended 2-4 weeks of antifungal therapy for non-homogenous leukoplakia to observe improvement in the lesion.^[12] Vander waal also stated that 2-4 week interval to observe the possible regression or disappearance of a white lesion after elimination of possible causative factors, seems an acceptable period of time.^[2]

Observing for the presence of candidal hyphae in cytospin smear before and after treatment and correlating the candida colonization with the salivary candida counts, 42% of cases showed presence of candidal hyphae before treatment and absence of candidal hyphae in all the cases following topical application of 1% clotrimazole for 2 weeks. (D1) Nada Vuckovic suggested that leukoplakias associated with conventional forms of smoking showed candidal hyphae more often than those associated with tobacco chewing. It is generally believed that the presence of candidal hyphae merely represents a superimposed infection.^[6] Candidal colonization of Leukoplakia is believed to be higher in smokers of tobacco than chewers of tobacco as smoking induces higher degree of epithelial keratinization favoring candidal colonization. Reduction in levels of Salivary IgA and depression of

Table 3: Candida colony counts before and after treatment

Colony count	No of Cases in pre treatment	No of cases in post treatment
0-600	1	35
800-2000	33	7
2000-4000	6	6
4000 -6000	6	2
6000 -8000	4	0

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polymorph nuclear leukocyte function also favor increased candidal colonization in smokers.^[13] Our study found that 64% of the subjects used Smoked tobacco which could be the reason for the high percentage of candidal hyphae observed before treatment.

Assimilating the observed results, 48% of cases showed improvement in staging *i.e.* got reversed to L1P0 stage; 12% cases got reversed to L2P0 from a higher stage and 40% of cases remained at L1P0 stage following treatment. Though there was post treatment reduction in the size of the clinical lesion in those 40% of cases (L1P0), there was no difference observed in staging following treatment. The regression in size of the lesion could not be documented in the form of staging because a lesion <2 cm however small, has to be graded as L1. Hence a pretreatment lesion which is 2 cm in size regressing to 0.5 cm post treatment still is graded as L1 [Table 4].

Warnakulasuriya found that Non homogeneous lesions carry a much higher risk of malignant transformation.^[1] In our study 14% cases of non-homogenous leukoplakia into homogenous leukoplakia following treatment. Most interesting observation in our study was the significant reduction in the size of the lesion and improvement in the clinical staging of leukoplakia before and after topical antifungal therapy thus reducing the changes of malignant transformation (D2). We also observed a significant reduction in candida colony count following the application of 1% w/v clotrimazole which directly correlated to clinical staging and candida colonization of the lesion which strongly signifies the role of Candida in Leukoplakia (D3).

Though candidal infection in leukoplakic lesions are mostly considered as a local association, the higher salivary colony counts estimated in most of the leukoplakia cases in our study provide a strong evidence of correlation of salivary candida level to tissue colonization.

The significant regression of the lesions and clinical presentation of the lesions also can be attributed partly to the Habit counseling performed before treatment. Even then, 48% of the cases improving to L1P0 stage along with reduction in Salivary Candidal count and complete elimination of candida hyphae emphasize the role of topical Clotrimazole antifungal therapy.

Maghu *et al.* have stated that a complete elimination of fungal hyphae could be achieved only by clotrimazole. Clotrimazole proved to be 100% successful in the reduction of clinical and histopathological features.^[14] An important observation was the reduction in the Salivary Candidal count and complete absence of Candida Hyphae after treatment with antifungal agent. Paired t test showed significant reduction in CFU with a p value of <0.001. The reduction in the candida colony count before and after application of antifungal therapy conforms with study results of Pragati *et al.*^[15] In a study conducted by Cao, OL cases found significant correlation with saliva culture results and concluded that candidal infection may be one of the most important factors inducing

dysplasia of epithelia and malignant transformation of oral leukoplakia thus emphasizing salivary candida culture as a routine procedure for patients with OL.^[16]

STRENGTH OF THE STUDY

Evaluation of the association of candidal hyphae in cytosmear as well as estimation of candidal colony count in saliva simultaneously before and after treatment with topical antifungal therapy has added up to the strength of the study. The present study showed 11 cases of non-homogenous leukoplakia, in which 7 cases turned into homogenous leukoplakia post treatment with application of 1% w/v clotrimazole antifungal therapy. There is a significant change in clinical staging of leukoplakia cases which strongly signifies the role of Candida in Leukoplakia.

Conversion of a Non-homogenous Leukoplakia to a Homogenous Leukoplakia thus reduces the potential for malignancy.

Topical Clotrimazole proves to be an economical, easily available, patient compliant and effective antifungal therapy necessarily recommended in management of Leukoplakia.

LIMITATION OF THE STUDY

Inclusion of more number of cases with different histopathological grading might result in more conclusive result of candidal association and the prognosis of pre malignant lesion, leukoplakia cases.

CONCLUSION

There are conflicting opinions regarding the presence and association of candida with malignant and premalignant lesions. Few authors believe candida does not prove to have a causal relationship and its presence in Leukoplakia is incidental and is only a colonizer of a pre-existing lesion under favorable environmental conditions. Others say candida may have a direct or indirect role in the carcinogenesis and specifically some *Candida albicans* biotypes might contribute more to oral carcinogenesis than others. Though the association of *Candida albicans* in the malignant transformation of potentially malignant lesions is not proven with strong evidences, the present study assessing the clinical staging of oral leukoplakia, presence of candidal hyphae in the lesion and salivary candidal colony count before and after treatment with antifungal therapy showed improvement in the clinical staging, elimination of the candida hyphae and significant reduction in the salivary colony counts in the lesions. High candida colony counts in saliva before treatment also suggests the possible correlation of candida with the clinical stage of leukoplakia. The possible role of Candida in progression of leukoplakia is evident from the significant reduction of the candidal colony count and improvement in the clinical staging after the application of 1% w/v clotrimazole. Based on our results, a conclusion can be drawn that topical application of 1% w/v clotrimazole antifungal agent on Oral Leukoplakia significantly reduces the candidal colony count in saliva as well as the candidal hyphe associated with the lesion and therefore results in improvement in clinical staging and arrest progression of the lesions.

Conflict of interest

None.

External funding source

None.

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Table 4: Comparison of pre and post treatment clinical staging

Staging before treatment	No of cases	Staging after treatment	No of cases
L1P0	20	L1P0	20
L2P0	8	L2P0	0
		L1P0	8
		L3P0	0
L3P0 or L1L2P1	16	L2P0	1
		L1P0	15
		L3P1	0
L3P1	6	L3P0	0
		L2P0	5
		L1P0	1

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