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Original Research Article

QUALITATIVE ANALYSIS OF CANDIDA ALBICANS AND KOILOCYTES IN ORAL POTENTIALLY MALIGNANT DISORDERS AND ORAL SQUAMOUS CELL CARCINOMA

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Abstract

Fungal and viral infections especially *Candida albicans* and Koilocytes (Human papilloma virus altered cells) in premalignant and malignant lesions when compared to normal mucosa might indicate the contribution of these microorganisms in carcinogenesis.

Aim: To assess the frequency of *Candida albicans* and koilocytes in different oral lesions; to compare results with the Control group and to study the clinical significance of *Candida* infection and Koilocytes in different oral lesions.

Materials and method: Study was performed in the Department of Oral Pathology and Microbiology after getting approval by the Ethical Committee of the institution. 30 patients for each of above mentioned clinically diagnosed lesions were randomly selected during 2008-2009. Control group of 30 patients {same for each lesion} was selected from patients who had to undergo minor oral surgical procedures. Smears were obtained from the selected site and were stained with PAS stain to check for presence or absence of Candida hyphae. Also, an incisional biopsy of the lesion was performed: one section was stained with H & E stain for koilocytes and other with PAS stain for presence or absence of Candidal hyphae.

Results: Incidence of *Candida albicans* positivity in smear was significantly higher in Oral squamous cell carcinoma group as compared to other groups ($p<0.05$). Presence of Koilocytes in these lesions was non- significant.

Conclusion: Linear relationship of Candida with the transformation of premalignant lesions and conditions to malignant lesions. Higher incidence of Koilocytes in premalignant and malignant lesions, when compared to normal mucosa, indicates the contribution of HPV viruses in carcinogenesis.

Keywords: Candida albicans, candidal hyphe, koilocytes, premalignant, carcinogenesis.

Introduction

Candida species have been considered as the most opportunistic infection in the world. Their disease prevalence has further increased in recent years that are generally due to an increase in a number of immunocompromised states of patients. *Candida* is commensal organisms. The predominant species isolated being *Candida albicans*.

Colonization of the human oral cavity by *C. albicans* usually indicates a saprophytic association with the host. However, localized *C. albicans* infection of mucosal membrane appear under certain pathologic conditions such as lichen planus and leukoplakia. Oral squamous cell carcinoma constitutes about 80-90% of the total malignancies occurring in the oral cavity (1).

The association between *Candida* species and oral cancer was first reported in the 1960s. The presence of *Candida albicans* is a common finding in the biofilm of premalignant lesions as well as in the normal oral cavity. (2).

Increased frequency of pre-cancerous lesions and cancers in the oral cavity has encouraged studies about etiology and pathogenesis of these lesions. All these suggested an important role of chemical carcinogens, radiation energy, chronic irritation, and viruses (3).

Sreejyothi et al. (4) and **Lima et al.** (5) suggested that specific types of Human papilloma virus (HPV) exhibited various distributions in lesions of the oral cavity. They demonstrated that HPV 6/11 were present in benign cases, while HPV 16/18 and HPV 31/33/51 were present in intraepithelial neoplasms and cancers.

HPV infection is generally seen in keratinocytes of the epidermis. Initially, viral particles pass from an eroded region of epithelium into cells in the basal layer. Where these stimulates the synthesis of regulatory proteins for viral DNA replication. Early viral genes stimulate cellular division in basal cells that leads to hyperplasia in upper layers of epithelium. Cells on the more superficial layers undergo nuclear degeneration and perinuclear cytoplasmic vacuolation, called koilocytosis. Histological demonstration of Koilocytes is considered as a hallmark of HPV infection.

The aim of this study was to investigate the presence of *C. albicans* and Koilocytes in tissue sections of specific oral lesions, since *C. albicans* and HPVs are thought to have a synergistic effect in the pathogenesis of benign and precancerous lesions and cancers of oral cavity, together with other etiological factors.

The present study was aimed to assess prevalence of occurrence of *Candida albicans* and koilocytes in different oral lesions such as Oral submucous fibrosis, Oral leukoplakia, Oral lichen planus and Oral squamous cell carcinoma, to statistically analyse the data and to assess the level of significance and to compare results with that of Control group and to study the clinical significance of *Candida* infection and Koilocytes in different oral lesions.

Materials & method

Necessary protocols were adhered to with regard to permission from ethical committee and patients were selected for this study after obtaining consent from them.

The prospective study was undertaken to probe frequency and degree of *C. albicans* and Koilocytes in various oral mucosal lesions. Lesions included in this study were Oral submucous fibrosis (OSMF), Oral leukoplakia (OL), Oral lichen planus (OLP) and Oral squamous cell carcinoma (OSCC). 30 patients for each of the above mentioned clinically diagnosed four lesions were randomly selected from Out Patient Department. Control group of 30 patients {same for each lesion} was selected

from patients who had to undergo minor oral surgical procedures.

After routine blood investigations, the most representative site was selected for the smear to check for presence or absence of Candidal hyphae. Smears were obtained by the thorough scraping of debris with a blunt metal spatula and fixed with spray fixative (95% Ethanol + 3% Glacial acetic acid). Also, an incisional biopsy of the lesion was performed under aseptic conditions and local anesthesia. Biopsy specimens were preserved in 10% neutral buffered formalin solution.

Smears were stained with Periodic acid Schiff stain and the biopsied tissues were processed and embedded in paraffin wax. Paraffin blocks were sectioned with a rotary semi-automatic soft tissue microtome into two sections of 5 μm thickness. One section was stained with Hematoxylin and Eosin stain and the other with Periodic acid Schiff stain.

The examination was carried out on Olympus BX51 Trinocular Light Microscope with provision for photomicrography.

C. albicans in smear was determined as present or absent. Results of PAS-stained smears were regarded as either absent (no hyphae) or present (hyphae found in the specimen). (6) PAS-stained tissue sections were similarly evaluated for the presence of Candidal hyphae. H & E stained sections were also observed for histological criteria in confirmation of diagnosis. In PAS-stained tissue sections, Candidal hyphae were characterized in the same pattern as that in the smear. **(Fig.1)**

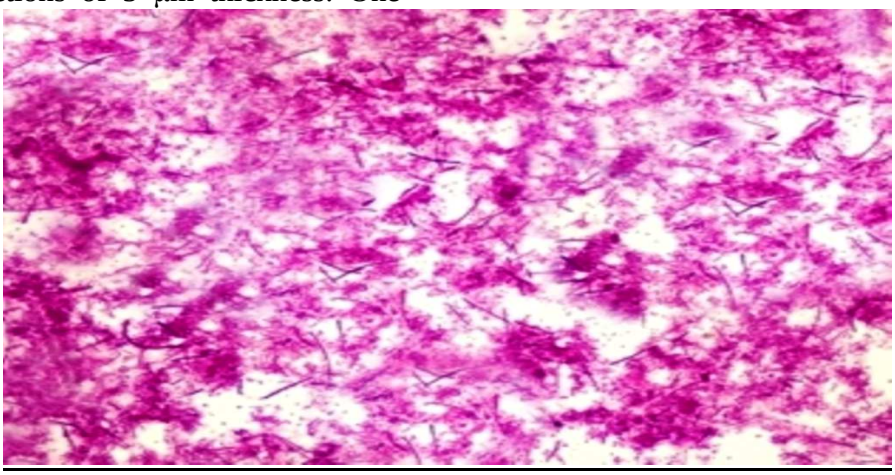

Fig.1: Photomicrograph showing magenta colored tubular hyphae of Candida albicans in PAS stained smear (X10)-original

In a histopathological examination of H & E stained tissue sections of above-mentioned lesions, the presence of koilocytes was evaluated under light microscopy. Koilocytes are squamous epithelial cells that have undergone specific cytopathic effect showing a number of structural changes, which occur as a result of infection of the epithelial cells by Human Papillomavirus only. (7) Koilocytosis or koilocytic atypia or koilocytotic atypia are terms used in histology and cytology to describe the presence of koilocytes in a specimen.

Koilocytes have the following cellular changes: Nuclear enlargement (two to three times normal size), Irregularity of the nuclear membrane, Nuclear Hyperchromatism and a clear area around the nucleus, known as a perinuclear halo.

Caution was taken to distinguish Koilocytes from other vacuolated cells. **(Fig.2)**

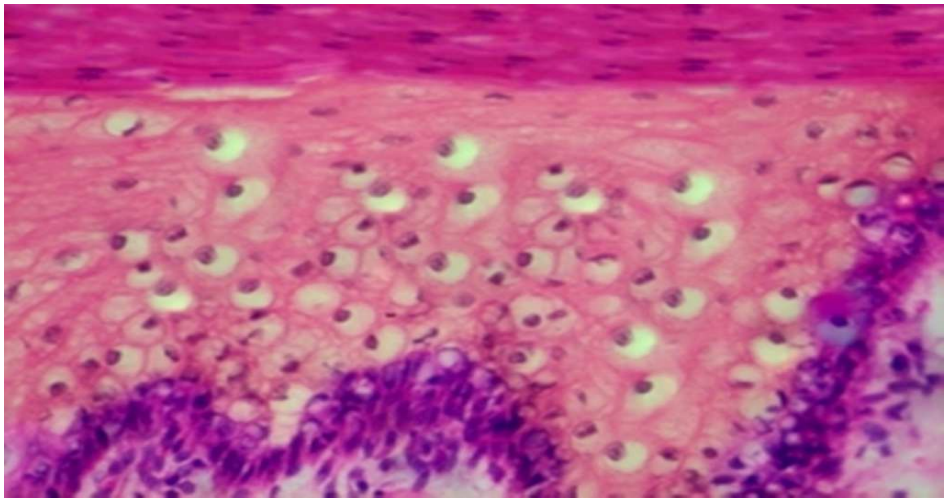

Fig.2: Photomicrograph showing Koilocytes in H&E stained section (X40)- original

Results were tabulated and subjected to **Chi-square test (χ^2)** for statistical analysis and level of significance i.e. *p*-value was noted. Results were found to be statistically significant in comparing the association of *C. albicans* in smear and biopsied tissue in potentially malignant disorders and oral squamous cell carcinoma i.e. *p*-value <0.05. While the presence of koilocytes in the same lesions was found to be statistically nonsignificant i.e. *p*-value > 0.05.

Results

The incidence of *C. albicans* positivity in smear was significantly higher in OSCC group as compared to OSMF, OLP, OL and Control group (p<0.05). Findings of *C. albicans* positivity in biopsy of the same cases followed a similar pattern as in smear. On comparing the presence of *C. albicans* in smear and biopsy, results were found to be statistically significant in OSMF and OL (p<0.05), whereas for other groups, it was not significant (p>0.05). It was also seen that for the occurrence of *C. albicans* in smear and biopsy when compared to Control group, results were nonsignificant in OSMF, OL and OLP cases, whereas, smear findings of OSCC were significantly higher (p=0.010). **(Table 1)**

Table 1: Comparison of *C. albicans* in Smear and Biopsy

S.No.	Group	Total No. of cases	In smear No.	In Biopsy No.	P
1.	OSMF	30	12	4	0.015
2.	Leukoplakia	30	16	7	0.018
3.	Lichen Planus	30	12	10	0.787
4.	Squamous cell CA	30	21	16	0.190
5.	Control group	30	5	0	0.153

On comparing the presence of Koilocytes in different Study groups, no statistical significance could be elicited, while positivity ranged from 30% to 53.3%.

Results were also not statistically significant when Study groups were compared with the Control group (p>0.05). **(Table 2)**

Table 2: Detection of Koilocytes in Biopsy

S.No.	Group	Total No. of cases	Number Positive	P
1.	OSMF	30	10	0.451
2.	Leukoplakia	30	16	0.071
3.	Lichen Planus	30	10	0.451
4.	Squamous cell CA	30	12	0.922
5.	Control	30	7	0.597

On detection of Candidiasis and Koilocytes in Study groups and Control group, *Candida albicans* in smear was the most common finding in all study groups except in Control group, in which the incidence of Koilocytes was most prevalent. Among all cases, evidence of *C. albicans* in biopsy was least common finding. **(Table 3)**

Table 3: Detection of Candidiasis and Koilocytes in study groups and Control group

S.No.	Group	Total No. of cases	Koilocytes		*Candida* in smear		*Candida* in biopsy	
			No. +ve	% +ve	No. +ve	% +ve	No. +ve	% +ve
1.	OSMF	30	10	33.3	12	32	4	13.3
2.	Leukoplakia	30	16	53.3	16	53.3	7	23.3
3.	Lichen Planus	30	10	33.3	12	32	10	33.3
4.	Squamous cell CA	30	12	40	21	70	16	53.3
5.	Control	30	7	23.3	5	16	0	0

Discussion

Candida albicans

C. albicans are members of normal oral flora predominant in the normal population and also can invade tissues to cause Candidal infection (8). Among all the species of *Candida*, *C. albicans* has been found as the most commonly encountered organism in general dental practice (9).

C. albicans lives with normal flora of the mouth, vaginal tract, and gut. In presence of pregnancy, oral contraception, antibiotic therapy, diabetes, steroid therapy, *etc.*, these become pathogenic and produce budding spores and elongated cells (pseudohyphae) or true hyphae with septate walls. These infect only outer layers of the epithelium of mucous membrane and skin and peels it away. Infected mucous membranes of mouth accumulate scales and inflammatory cells that develop into characteristic white or white-yellow, curdy material (10).

The aim of our study was to probe if there is any significant association of *C. albicans* infection with certain specific oral mucosal lesions. This study has also been done to determine the type of lesion that is most frequently associated with it that may help clinicians to treat the patient not only for a particular lesion that is present, but also to treat infection by *C. albicans* so as to reduce its potential to malignant transformation.

On statistical analysis, we found that the incidence of *C.albicans* positivity in smear was significantly higher in OSCC group. Maximum positivity was seen in

OSCC group and minimum positivity in Control group.

Results of our study were in accordance with **Supriya et al.** (10) who explored the association of *C. albicans* (in smear as well as in biopsy) and various types of mucosal lesions. They concluded that *C. albicans* is present in potentially malignant oral mucosal lesions (in 25% of leukoplakia cases, in 44.44% of lichen planus cases, and in 100% of squamous papilloma cases), with an increasing incidence in lesions with malignancy.

The results of our study were also found to be similar to that of **Archilla et al.** (11), **Sarkar et al.** (12) and **Shree et al.** (13).

In our study, results for *C. albicans* in biopsy showed 10% positivity for OSMF that was minimum. In OL 26.7%, in OLP 33.3% and in OSCC, 50% of cases were positive for *C.albicans* in the biopsy. Control group showed significantly lower evidence of *C. albicans* in biopsy as compared to other groups ($p<0.05$). Results were similar to those in smears except for prevalence of *C. albicans* in biopsy cases of OL and OLP, as, in smears, number of OL patients affected by *C. albicans* were more than the number of OLP patients, affected by the same organism.

According to our study, *C.albicans* in smears was found to be in increasing incidence from OSMF, OL, OLP, and OSCC. These results clearly indicated that *C. albicans* might have carcinogenic potential. A similar conclusion was derived by **Archila et al.** (11) who performed a study in which a model of oral mucosal carcinogenesis using water-soluble carcinogen 4-nitroquinoline-1-oxide (4NQO) was combined with a model of oral mucosal candidosis to examine the ability of *C. albicans* to promote the development of neoplasia in ^{the} suitably initiated epithelium. Development of carcinoma indicated that particular strain of *Candida* used, had a similar ability to promote neoplastic changes.

Our study was contrary to findings of **Daftary et al.** (14) in which they suggested that there is no significant association between *Candida*-positive cases and atypia found.

In our study, the difference was seen in the results for the presence of *C. albicans*, obtained by taking smear and biopsy of the same patients. After viewing all laboratory procedures, we came to the conclusion that the difference might be due to some loss of *Candida* during some laboratory procedures. Our view for the difference seen in relation to presence of *C. albicans* in smear and biopsy was similar to a study conducted by **Skoglund et al.** (15) in which they have mentioned that noninvasive hyphae and fungi could be diagnosed in smears and in culture, but could be lost during laboratory handling of specimen, leading to a negative result in histopathological examination or vice versa.

Koilocytes

Human papilloma viruses (HPV) included under DNA viruses are present in humans with normal oral mucosa. These viruses may cause benign tumors in normal appearing mucosa and even some of them may turn malignant also. They may be responsible for Koilocytes, a characteristic feature of koilocytic dysplasia caused by HPV (16).

There are 16 genotypes of HPV which have been isolated in oral lesions and can be categorized into low risk (6, 11, 13, 32), medium risk (31, 33, 35, 51) and high risk (16) according to the grade of correlation with certain malignant neoplasms. Low-risk HPV is considered to be predominantly associated with benign lesions (condyloma accuminatum, common wart, squamous papilloma, focal epithelial hyperplasia). Medium and high-risk HPVs

are involved in etiology and progression of pre-malignant and malignant lesions (17).

HPV genome encodes DNA sequences for 6 early (E) proteins associated with viral gene regulation and cell transformation and 2 late (L) proteins which form the shell of the virus. Two most important HPV proteins are E6 and E7. These act synergistically to transform cells. These oncoproteins stimulate cell proliferation by activating cyclins E and A (18).

According to literature, HPV was 2-3 times more in precancerous oral mucosa and 4.7 times more in Oral carcinoma than in normal mucosa.

In our study, in H & E sections, HPV infection positivity ranged from 30% to 53.3% but no statistical significance was observed, when results were compared among different groups ($p>0.05$).

Our results were similar to **Purwaningsih et al.** (19). in which 60% of OL cases were affected by HPV that was higher as compared to other lesions. Our study was also in accordance with **Jalauli et al.** (20) and **Syrjanen et al.** (21).

Results of our study were contrary to **Mork et al.** (22) who suggested that HPV may be a risk factor for OSCC.

Based on statistical analysis, there was no significant association of HPVs with the progression of Oral carcinoma. In our study, Koilocytes in OL was slightly higher when compared to OSCC that again put the role of HPV in the progression of malignancy in doubt.

However, we also observed a higher incidence of Koilocytes in OSMF, OL, OLP, and OSCC when compared to the Control group. Hence, the role of HPV in carcinogenesis cannot be ruled out. Many investigators have proposed that the presence of HPV is probably essential for early events, in malignant transformation, consistent with "Hit and Run" hypothesis (23). So, we may presume that HPV plays a role in the initiation of dysplastic changes, but it is not a requisite for progression to malignancy.

Conclusion

The present study suggests a direct linear relationship of the Candidal carriage and koilocytic dysplasia with the transformation of various premalignant lesions and conditions to malignant oral mucosal lesions. Considering the limitations of light microscopy (only 80% accuracy) (24), studies with larger samples and better-specialized methods with advanced equipment, might increase the accuracy of findings and also define the precise role of *C. albicans* and HPVs in oral neoplasia. This would help clinicians to define the precise role of Candida and HPV in oral neoplasia, particularly to examine the state of a gene, its expression and its interaction with oncogenes, so as to treat infection at an early stage and with better prognosis.

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