



Association Of Fungal And Viral Entities With Specific Personal Habits – An Original Study

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Abstract

Introduction: Fungi and HPV infection is generally seen in the keratinocytes of the epidermis. Also, habits like tobacco and alcohol produce a significant synergistic effect on the incidence of Candida and HPV infection. In this study, we aimed to investigate the role of tobacco and alcohol consumption on the occurrence of Candidal hyphae and the presence of koilocytes in tissue sections of oral potentially malignant disorders and oral squamous cell carcinoma.

Aim & Objectives: To assess prevalence and correlate occurrence of specific habits on *Candida albicans* and koilocytes in different oral lesions such as Oral submucous fibrosis, Oral leukoplakia, Oral lichen planus and Oral squamous cell carcinoma.

Materials And Method: After proper history taking and routine blood investigations, the most representative site was selected for smear to check for presence or absence of Candidal hyphae. Also, in histopathological examination of H & E stained tissue sections of above mentioned lesions, presence of koilocytes was evaluated under light microscopy.

Result & Conclusion: Prevalence of oral candidal colonization and koilocytes were higher in the smokers and tobacco chewers in comparison to the nonusers of tobacco. Also, their prevalence was more associated with the subjects with oral squamous cell carcinoma and oral submucous fibrosis among the oral potentially malignant disorders.

Keywords: synergistic effect, Candidal hyphae, koilocytes

Introduction

The prevalence of diseases caused by Candida species has increased in recent years. It is considered as one of the major fungal pathogens of immunocompromised patients. These fungi are commensal organisms found in approximately 40%

of individuals, the predominant species isolated being *Candida albicans*. *C. albicans* has the potential to infect virtually any tissue within the body, however, it is predominantly found on oral mucosa. [1] Possible association between Candida species and

oral neoplasia was first reported in the 1960s with later reports suggesting a link between the oral mucosal presence of *C. albicans* and the development of oral squamous cell carcinoma (OSCC). [2] Cell surface hydrophobicity, adhesion, hyphae formation and protease secretion are known to contribute to the virulence of *C. albicans*.

Colonization of the human oral cavity by *Candida albicans* often occurs and usually leads to a saprophytic association with the host. Thus, a number of oral mucosal lesions such as oral Lichen planus, oral Leukoplakia, and oral submucous fibrosis are sometimes invaded by yeast species, especially *C. albicans*. [3] Similarly, elderly denture wearers may suffer from candidiasis, an infection that may give rise to denture stomatitis. Various environmental as well as nutritional factors may regulate yeast to hyphal form morphogenesis in *C. albicans*.

Apart from *Candida albicans*, in recent years, the frequency of pre-cancerous lesions and cancers in the oral cavity has prompted studies as to the etiology and pathogenesis of these lesions. It is suggested that chemical carcinogens, radiation energy, chronic irritation and viruses play an important part in their etiology.

Viruses generally infect larynx, conjunctiva and oral cavity. [4] Woods et al and Miller et al suggested that specific types of human papilloma virus (HPV) exhibited various distributions in the lesions of the oral cavity. In their studies, they demonstrated that HPV 6/11 was present in benign cases, while HPV 16/18 and HPV 31/33/51 were present in intraepithelial neoplasms and cancers. [5]

infection is generally seen in the keratinocytes of the epidermis. Initially, viral particles pass from an eroded region of the epithelium into the cells in the basal layer. After penetrating into the cells of the basal layer, the virus stimulates the synthesis of regulatory proteins for viral DNA replication. Early viral genes stimulate cellular division in basal cells. As a result of this stimulation, excess cellular division reveals itself as hyperplasia in the upper layers of the epithelium. The cells on the more superficial layers undergo nuclear degeneration and perinuclear cytoplasmic vacuolation, called koilocytosis. [6] As the basal cell differentiates and proceeds towards the upper layers, viral DNA replication is stimulated, synchronized and stabilized

by cellular DNA replication. As the infected keratinocytes are shed, the virus infects other areas. (Human papilloma viruses (HPVs) may cause papillomas (benign epithelial growths), or skin and genital warts. [7] [3]

It has been known that various environmental factors like tobacco, alcohol and its combined use may regulate yeast to hyphal form morphogenesis in *C. albicans*.

The combined exposure to tobacco and alcohol produced a significant synergistic effect on the incidence of *Candida* and HPV infection. [8]

In this study, we aimed to investigate the role of tobacco and alcohol consumption on the occurrence of *Candidal* hyphae and the presence of koilocytes in tissue sections of oral potentially malignant disorders and oral squamous cell carcinoma since *Candida* and HPV are thought to have a synergistic effect in the pathogenesis of benign and precancerous lesions and cancers of the oral cavity, together with other etiological factors.

Aims & Objectives

1. 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.
2. To correlate specific habits (tobacco chewing, tobacco smoking, alcohol and their combined use) with the prevalence of *Candida albicans* and koilocytes in different oral lesions such as Oral submucous fibrosis, Oral leukoplakia, Oral lichen planus and Oral squamous cell carcinoma.

Materials & method

The prospective study was undertaken to correlate specific habits (tobacco chewing, tobacco smoking, alcohol and their combined use) with the prevalence of *Candida albicans* and koilocytes in different oral lesions such as 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 of Oral Medicine and Radiology during period from two years. Control group of 30 patients

{same for each lesion} was selected from patients who had to undergo minor oral surgical procedures.

After proper history taking and routine blood investigations, the most representative site was selected for smear to check for presence or absence of Candidal hyphae. Smears were obtained by thorough scraping of debris with a blunt metal spatula and fixed with spray fixative (95% Ethanol + 3% Glacial acetic acid). Also, 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 according to method described by Bancroft JD *et al.* and the biopsied tissues were processed and embedded in paraffin wax. [9] 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, according to procedures described by Culling CFA *et al.* and Bancroft JD *et al.* respectively. [10]

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). PAS stained tissue sections were similarly evaluated for 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 smear.

In histopathological examination of H & E stained tissue sections of above mentioned lesions, 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. 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.

Presence or absence of Candida albicans and koilocytes were then correlated with their personal habits like tobacco chewing, tobacco smoking, alcohol and their combined use.

Results

Table – 1: Distribution of Subjects according to Lesion Type

Lesion Type	No.	%
Leukoplakia	30	23.1
Lichen planus	30	23.1
Oral submucous fibrosis	30	23.1
Squamous cell carcinoma	30	23.1
Normal	30	23.1
Total	150	115.5

In our study, each of the four pathological conditions (leukoplakia, lichen planus, oral submucous fibrosis, and squamous cell carcinoma) have the same number of cases, which is 30, and they each make up 23.1% of the total cases.

Table – 2: Distribution of Subjects according to Baseline Characteristics

Baseline Characteristics		No.	%
Age	16 - 25 yr	9	6.9
	26 - 35 yr	26	20.0
	36 - 45 yr	38	29.2
	46 - 55 yr	31	23.8
	56 - 65 yr	18	13.8
	66 - 75 yr	8	6.2
	Mean±SD	44.05±12.62	
Sex	Male	78	60.0
	Female	52	40.0
Site of Lesion in mouth	Alveolar	19	14.6
	Buccal Mucosa	85	65.4
	Floor of mouth	5	3.8
	Gingiva	4	3.1
	Palate	2	1.5
	Tongue	15	11.5
Occurrence	NA	10	7.7
	Bilateral	91	70.0
	Unilateral	29	22.3
Habit	None	16	12.3
	Tobacco chewing	44	33.8
	Tobacco smoking	22	16.9
	Alcohol	3	2.3
	Combined	45	34.6
Total		130	100.0

The above table presents baseline characteristics of the study participants, including their age, sex, site of lesion in the mouth, occurrence, and habit. The table reports the number and percentage of participants in each category.

Table – 3: Distribution of Subjects according to Fungal and Viral Elements

Fungal and viral	No.	%
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elements		
Candida in smear	79	53.1
Koilocytes in Biopsy	62	40.0
Total	150	100.0

The table lists two types of elements, including Candida in smear and Koilocytes in Biopsy. For each element, the table shows the number of cases and the percentage of cases out of a total of 150 cases.

Overall, the table shows that both Candida and Koilocytes were present in the sample or dataset, although Candida was found more frequently than Koilocytes.

Table – 4 : Association of Habits with Fungal and Viral Elements with various Lesion Types

Lesion Type	Habit	Candida in smear				Koilocytes in Biopsy			
		Negative		Positive		Negative		Positive	
		No.	%	No.	%	No.	%	No.	%
Leukoplakia	None	2	50.0%	2	50.0%	1	25.0%	3	75.0%
	Tobacco chewing	2	25.0%	6	75.0%	4	50.0%	4	50.0%
	Tobacco smoking	3	50.0%	3	50.0%	3	50.0%	3	50.0%
	Alcohol	0	0.0%	1	100.0%	0	0.0%	1	100.0%
	Combined	5	45.5%	6	54.5%	6	54.5%	5	45.5%
	significance	chi sq=1.97, p=0.741				chi sq=1.97, p=0.742			
Lichen planus	None	2	40.0%	3	60.0%	3	60.0%	2	40.0%
	Tobacco chewing	2	22.2%	7	77.8%	7	77.8%	2	22.2%
	Tobacco smoking	3	75.0%	1	25.0%	3	75.0%	1	25.0%
	Alcohol	2	100.0%	0	0.0%	1	50.0%	1	50.0%
	Combined	7	70.0%	3	30.0%	6	60.0%	4	40.0%
	significance	chi sq=7.48, p=0.113				chi sq=1.18, p=0.882			
Oral submucous fibrosis	None	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Tobacco chewing	10	55.6%	8	44.4%	9	50.0%	9	50.0%
	Tobacco smoking	0	0.0%	2	100.0%	2	100.0%	0	0.0%
	Alcohol	0	0.0%	0	0.0%	0	0.0%	0	0.0%

	Combined	6	60.0%	4	40.0%	8	80.0%	2	20.0%
	significance	chi sq=2.50, p=0.287				chi sq=3.73, p=0.155			
Squamous cell carcinoma	None	0	0.0%	1	100.0%	0	0.0%	1	100.0%
	Tobacco chewing	4	50.0%	4	50.0%	2	25.0%	6	75.0%
	Tobacco smoking	2	25.0%	6	75.0%	8	100.0%	0	0.0%
	Alcohol	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Combined	4	30.8%	9	69.2%	8	61.5%	5	38.5%
	significance	chi sq=1.79, p=0.617				chi sq=10.93, p=0.012			
Normal	None	4	66.7%	2	33.3%	4	66.7%	2	33.3%
	Tobacco chewing	0	0.0%	1	100.0%	1	100.0%	0	0.0%
	Tobacco smoking	2	100.0%	0	0.0%	2	100.0%	0	0.0%
	Alcohol	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Combined	1	100.0%	0	0.0%	0	0.0%	1	100.0%
	significance	chi sq=3.65, p=0.302				chi sq=3.65, p=0.302			

The table shows the number and percentage of patients with negative and positive Candida results for each habit and lesion type. Chi-square tests were performed to determine the significance of the associations between habit and Candida results for each lesion type.

For each lesion type, the table shows the distribution of Candida in smear by habit, including none, tobacco chewing, tobacco smoking, alcohol, and combined (i.e. more than one habit).

Discussion

Candida albicans usually leads to a saprophytic association with the host tissues has a positive role in initiation and progression of pre-cancerous lesions and cancers in the oral cavity. Also, various forms of human papilloma virus (HPV) exhibited various distributions in the lesions of the oral cavity. Apart from these, various environmental factors including tobacco, alcohol and its combined use may induce yeast to hyphal form transformation in C. albicans and HPV. So, to find the role of tobacco and alcohol consumption on the occurrence of Candidal hyphae and the presence of koilocytes (HPV infected cells) in tissue sections of oral potentially malignant disorders and oral squamous cell carcinoma, we conducted a study which included 30 subjects of each of the four pathological conditions (leukoplakia,

lichen planus, oral submucous fibrosis, and squamous cell carcinoma) that constituted 23.1% each of the total population. Similarly, a prevalence of 30% of oral potentially malignant disorders was found in the population when the pilot study was conducted by Kumar S et al 2021. [11]

In our study, regarding age, our study showed that the majority of participants were in the age range of 36-45 years (29.2%), followed by 26-35 years (20%), 46-55 years (23.8%), and 56-65 years (13.8%). The mean age of participants was 44.05 years, with a standard deviation of 12.62. In terms of gender, 60% of participants were male and 40% were female. The most common site was found to be the buccal mucosa (65.4%), followed by tongue (11.5%), alveolar (14.6%), floor of mouth (3.8%), gingiva (3.1%), and palate (1.5%). 70% of lesions were bilateral, 22.3%

were unilateral. The habit of participants was also recorded, with 34.6% reporting a combined habit of tobacco chewing, tobacco smoking, and alcohol consumption, followed by tobacco chewing (33.8%), tobacco smoking (16.9%), alcohol consumption (2.3%), and no habit (12.3%).

Overall, these demographics provide a comprehensive overview of the baseline characteristics of the study participants, which may be useful for understanding the distribution of various factors in the study population. Almost same demographics were obtained by a study conducted by Ashwanirani SR *et al* 2020 in which out of total 130 patients, 63.9% were males and 36.1% were females. The most common oral PMD found was tobacco pouch keratosis, then oral submucous fibrosis and lichen planus minimum of smokers palate, actinic cheilitis and erythroplakia. The most common site affected was buccal mucosa and smoking and use of smokeless form of tobacco were the most common etiological factors in their study. [12]

On evaluating the distribution of Subjects according to fungal and viral elements, 79 cases, representing 53.1% of the total cases, were positive for Candida in smear, which is a type of fungal element. Additionally, 62 cases, or 40% of the total cases, showed Koilocytes in Biopsy, a type of viral entity. Same findings were found in studies conducted by Sankari SL *et al* 2019 and Lohakpure N *et al* 2019 [13] [14] in which they found a significant association of oral occurrence of candida in oral potentially malignant disorders in comparison to healthy cohorts was observed. They also suggested that Candidal species may be potent risk factor for transition of OPMD to oral Squamous Cell Carcinoma. Also similar conclusion was achieved by Purwaningsih NMS *et al* 2017 in which they suggested HPV were increased following degree of malignancy that HPV koilocytes were increased following degree of malignancy. [15]

Further in our study, on studying the association of Habits with Fungal and Viral Elements with various lesion types, the results showed that among patients with leukoplakia, the proportion of positive Candida results was higher among those who chew tobacco, smoke tobacco, or use alcohol compared to those with no habit. However, the chi-square test did not find a significant association between habit and

Candida results for leukoplakia. Similar results were obtained by Feller L *et al* 2012 and Shang Q *et al* 2020 in which they suggested that there is little evidence to support a causal relationship either between HPV infection and oral leukoplakia or between HPV-infected leukoplakic keratinocytes and their carcinomatous transformation. [16] [17]

For lichen planus, there was a higher proportion of positive Candida results among patients who chew tobacco, and a higher proportion of negative results among those who smoke tobacco. However, the chi-square test did not find a significant association between habit and Candida results for lichen planus either. Lubis I *et al* 2020 and Ashwinirani SR *et al* 2020 also noted in their study, a high prevalence of positive HPV, in case groups (96.4%). Also a high prevalence of positive HPV on betel quid chewers in Samosir island Indonesia, both in subjects with oral potentially malignant disorders and control group was found in their study. [18]

For oral submucous fibrosis, the results showed a higher proportion of positive Candida results among patients who smoke tobacco compared to those who chew tobacco or have no habit. However, the chi-square test did not find a significant association between habit and Candida results for oral submucous fibrosis. Similar results were concluded by Alanazi L *et al* 2019 in which they suggested that cigarettes may interact with *C. albicans* to promote their pathogenesis, which may increase the risk of oral candidiasis in cigarette users. [19] Also, Smoking and use of smokeless form of tobacco were found to be the most common etiological factors in Oral potentially malignant disorders as suggested by Ashwinirani SR *et al* in their study conducted in 2020. [12] Thus, it is suggested that *C. albicans* plays an important role in the etiology of precancerous lesions of the oral mucosa as well as in the dysplastic and malignant transformation of oral lesions. The ability of Candida to produce carcinogens such as nitrosamines and acetaldehydes, and the induction of proinflammatory cytokines, can be potential risk factors in the development of oral cancer.

For squamous cell carcinoma, the proportion of positive Candida results was higher among patients who smoke tobacco compared to those who chew tobacco or have no habit. However, the chi-square test did not find a significant association between

habit and Candida results for squamous cell carcinoma either. Thus, there is evidence that Candida is the direct cause of oral cancer probably only when the infection is chronic, deep, and associated with risk factors such as tobacco and alcohol, etc.

Finally, for normal patients, the proportion of positive Candida results was higher among those who smoke tobacco compared to those who have no habit. However, the chi-square test did not find a significant association between habit and Candida results for normal patients. Same conclusion was obtained by Darwazeh AMG *et al* in their study conducted in 2010 in which the prevalence and density of oral candidal colonization were both higher in the smokers compared to the nonsmokers. [20]

Further the results showed that the presence of Koilocytes is significantly associated with Squamous Cell Carcinoma (SCC) ($p=0.012$) and there is more trend towards an association with Oral Submucous Fibrosis (OSMF) and tobacco smoking among oral potentially malignant disorders ($p=0.155$). However, there was positivity but no significant association was found for Leukoplakia, Lichen Planus, or Normal oral mucosa. Similarly, Sumithrarachchi SR *et al* 2021 conducted a study in which they concluded that occurrence of OPMDs and oral cancer increases with increased use of BQ, smoking and alcohol.[21] and Kumar R *et al* 2015 suggested that tobacco chewing and alcohol consumption may act as risk factors for hr-HPV infection in HNCs from the North-East region of India and they also suggested that alcohol, tobacco and HPV infection act synergistically or complement each other in the process of head and neck carcinoma development and progression. [7] Thus, overall it is suggested that smoking, in combination with other factors, could enhance oral candidal and HPV growth in potentially malignant oral lesions especially oral submucous fibrosis and collectively constitutes a significant risk for the initiation and progression of oral carcinogenesis.

Conclusion

Within the confines of our study, prevalence of oral candidal colonization and koilocytes were higher in the smokers and tobacco chewers in comparison to the nonusers of tobacco. Also, their prevalence was more associated with the subjects with oral squamous cell carcinoma and oral submucous fibrosis among

the oral potentially malignant disorders. However, the difference was not statistically significant. Additional studies with larger sample size and more accurate methods are needed to examine the effect of habits on oral candida and HPV prevalence in oral potentially malignant disorders and oral squamous cell carcinoma.

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