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Analysis of oral candida in leukoplakia and tobacco pouch keratosis cases associated with smokeless tobacco

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Abstract
Aim: To evaluate and co-relate the prevalence of *Candida* species, salivary flow rate and buffer capacity in oral cavity of subjects with leukoplakia and tobacco pouch keratosis.
Material and Methods: The present prospective study was conducted among 20 cases of Leukoplakia, 20 Tobacco Pouch Keratosis and 40 Healthy controls. Sabouraud's Dextrose agar for the identification of *Candida* and Hi Crome *Candida* Differential Agar for the species identification of *Candida* was used. Calculation of Salivary Flow rate, pH, Buffer Capacity was done with Saliva Check Buffer Kit (GC).
Results: The prevalence of *Candida* carriage obtained was considerably greater in the study group associated with smokeless tobacco use. Statistically significant values were obtained between the Salivary Flow rate, pH and Buffer capacity on comparison among the study groups and control groups.
Conclusion: Present study indicated Candida prevalence was seen more in study group in comparison to healthy controls indicating to increase *Candida* in smokeless tobacco users.

Keywords: candida, smokeless tobacco, leukoplakia, tobacco pouch keratosis

Introduction

The effect of smokeless tobacco has always been ignored in context to potentially malignant disorders in comparison to smoking tobacco [1, 2]. Smokeless tobacco leads to leukoplakia, tobacco pouch keratosis, OSMF, periodontal infection, delayed wound healing and dental caries [3].

Smokeless Tobacco comes in two principal designs i.e. Snuff and Chewing Tobacco. Tobacco chewers mainly position chewing tobacco mostly in the buccal vestibule making site vulnerable to develop lesion. Researchers have pointed out it to as a Chow or Quid of chewing tobacco. The quid stick in the oral cavity for hours, and the saliva gets mixed with tobacco juice. These way chemical carcinogens in smokeless tobacco are released in the oral cavity that is Polynuclear Aromatic Hydrocarbons Usually benzo [a] pyrene, polonium 210 and N- nitrosamines, other chemicals include Radium 266 and Lead 210 which forms adducts and cause DNA damage and ultimately leads to malignant transformation [4].

Investigations in experimental animals revealed that benzo (a) pyrene and one of its metabolites leads to oral and perioral skin cancer [5]. Nicotine one of the principal constituents exerts a negative reaction at the cellular level by altering metabolism and invades the contacts between tissues, which advances the structure of cancer, altering cellular changes. It also affects the oral cavity microflora, by increasing the number of destructive bacteria and pathogenic fungi [6].

Tobacco Pouch Keratosis is a white lesion at the site which comes in contact in tobacco continuously, the lesion appears thickened and leathery and Oral Leukoplakia is the utmost potentially malignant disorder of oral mucosa which is characterized as a predominantly white lesion of the oral mucosa that cannot be described as any other definable lesion [7, 8].

The present prospective study to evaluate Candida species and different non-Candida albican candida *species* identification, assessment of Salivary Flow Rate and Buffer Capacity among subjects with Leukoplakia, Tobacco Pouch Keratosis associated with smokeless tobacco in comparison to healthy controls.

Material and Methods

The present prospective study was among 20 clinically diagnosed with Leukoplakia, 20 diagnosed with Tobacco Pouch Keratosis and 40 healthy controls subjects. The structure and purposefulness of the study was elaborated to each patient. An informed consent form was taken. SDA was used to identify *Candida* among the groups and then the Hi Crome Media Candida differential agar was used to further evaluate various strains of *Candida* species among the subjects.

Including criteria

The cases not smoking >20 years or never smoked, chewing tobacco for at least 5 years, not taking any medicine (antibiotic /fungal/corticosteroid) for 6 months, with no systemic conditions, not wearing any kind of prosthesis and absence of decayed teeth

The salivary samples were collected between 9 -11.30 a.m. in both the study and control groups. The salivary flow, pH, and buffering capacity were measured using "Saliva-check BUFFER kit (FIG 3)" (in vitro test for pH and Saliva Buffering Capacity) manufactured by GC Corporation. The kit is provided with a pH strips which measures the saliva collection cups, saliva dispensing pipette and buffer test

strips.

FU

For CFU the samples were taken by oral rinse technique, subjects were asked to rinse with 10 ml saline and expectorate, then 100µL was taken and samples were inoculated on the plate and were incubated for 48 hours at 37°C in the incubator and later colonies were calculated under the colony counter and speciation of *Candida* was done on the basis of distinctive morphological characteristics.

Statistical analysis

The data was collected and subjected to statistical analysis using SPSS version 24.

Result

Total subjects included in the present study were in the age range from 25-60 years. In study groups we found different species in the following descending order i.e. *tropicalis* > *C.albicans* > *C.glabrata* > *C.krusei.* Data indicates that more number of *Candida* is present more in study groups in comparison with control groups (table 1).

Subjects with Leukoplakia and Tobacco pouch keratosis associated with smokeless tobacco, candida carriage rate was found to be significantly increased and this increase in candida carriage can be related to the use of smokeless tobacco (table 2).

Statistical Co relation of CFU with Salivary flow rate, pH and buffer capacity was obtained by applying Pearson test and on comparing CFU among each group with Salivary flow rate, pH and buffer capacity we obtained statistically significant value i.e p≤ 0.01 in Leukoplakia group and in Tobacco pouch keratosis group p≤ 0.03.While on comparing CFU with salivary flow rate and Buffer capacity, p≤0.01 with pH. Among Healthy controls no significant relationship was obtained (table 3). The data indicates that Candida carriage rate in the oral cavity is influenced by the salivary flow rate, pH and buffer capacity. Statistically significant value obtained among study groups can be related to use of smokeless tobacco.

Table 1: Prevalence of Candida species obtained among groups on Hi Crome agar were identified on the basis of colour and morphology

Candida species identified	Study Group			Control Group
	Leukoplakia	Tobacco pouch keratosis	Total	
C.albican	20.0 %	31.0%	51%	31.9%
C.glabrata	31.1%	26.2%	57.3%	23.6%
C.krusie	4.4%	0.0%	4.4%	2.8%
C.tropicalis	44.4%	42.9%	87.3%	41.7%

Table 2: Comparison of prevalence of CFU among study groups and control group

Subjects		CFU/ml
Study group	Leukoplakia	p ≤ 0.01
	Tobacco pouch keratosis	p ≤ 0.01
Control group		p ≤ 0.01

Table 3: Correlation between CFU & Salivary Flow rate, and CFU & Buffer capacity

Group		CFU and Salivary flow rate		CFU and Buffer capacity	
		r value	p value	r value	p value
Study group	Leukoplakia	-0.58	<0.01*	-0.56	<0.01*
	Tobacco pouch keratosis	0.39	0.03*	0.42	0.03*
Control group		-0.08	0.91	-0.04	0.92

*statistically significant

Discussion

In the present study we have obtained statistically significant results for the presence of *Candida* among study groups who were associated with smokeless tobacco use (Leukoplakia and Tobacco pouch keratosis) on comparing with healthy controls who were not associated with use of smokeless tobacco. The obtained results can be explained on the basis as smokeless tobacco releases Nitrosamines in the oral cavity which changes the oral cavity microflora, increasing the number of harmful bacteria and pathogenic fungi and longer duration use of smokeless tobacco leads premalignant disorders e. g Leukoplakia, pathologic variations in the mucous membrane or tongue and tumor, it also decreases Salivary pH and increases in phosphorus ion concentration resulting in the more acidic environment and leading to the dryness of the mouth –Xerostomia, all the above mentioned factors increases the accumulation of *Candida* in the oral cavity [6], moreover the subjects included in the study group were healthy otherwise.

On the basis of above findings candida in the present study can be concluded as a superimposed infection in a lesion, which makes the lesion more vulnerable for malignant change by showing phenotypic change from yeast to hyphae form and producing carcinogen like nitrosamines, at the end leading to initiation of cancer development [7].

There are studies in literature which says opposite and similar to the result as found in our study; for eg. Roed Peterson *et al*. 1997 and Daftary *et al*. 1972 [9] concluded etiological role of *Candida* infection to be more significant. Bancozy (1977) [10] observed statistically significant results that *Candida albicans* infections acts as an etiological factor in malignant conversion of leukoplakia.

Other finding of the study is the prevalence of Non *Candida albican Candida* species among study groups mainly *C.tropicalis*. Studies in favour e.g Connolly *et al*. [11] explained similar results among smokeless tobacco users.

Increase in the Non *Candida albican Candida* as observed in our study was observed by Chakraborty *et al*. [12], who concluded *Candida tropicalis* is ubiquitous yeast in Asia, similarly the study by Sonia Silva *et al*. [13] concluded that *Non Candida albican Candida* species like *Candida glabrata, Candida tropicalis* are now commonly found as human pathogens.

Conclusion

The presence of Non-Candida *albican Candida* can be explained on the basis of new medical cures, the upsurge in invasive medical measures and the use of wide-ranging spectrum antibiotics, poor oral hygiene. However, there are significant variations in studies of *Candida* species,

depending on the geographical region and patient group, with some being more predominant in *Non-Candida albican Candida* species, and some with *C. albicans* in certain countries.

References

1. Centers for Disease Control and Prevention. Cigarette smoking- attributable mortality and years of potential life lost - United States. 1990. MMWR Morb Mortal Wkly Rep. 1993; 42:645-9.
2. US Department of Health and Human Services. Reducing the health consequences of smoking: 25 years of progress, a report of the surgeon general. Washington, DC: US Department of Health and Human Services. DHHS publication no. (CDC), 1989, 89-8411.
3. American Academy of Periodontology position paper. Tobacco use and the periodontal patient. J Periodontol. 1996; 67:51-6.
4. Main JH, Lecavalier DR. Smokeless tobacco and oral disease. J Can Dent Assoc. 1988; 54:586-91.
5. Hoffmann D, Brunnemann KD, Prokopczyk B, Djorkjevic MV. Tobacco-specific N- nitrosamines and Areca-derived N-nitrosamines: chemistry, biochemistry, carcinogenicity, and relevance to humans. J Toxicol Environ Health. 1994; 41:1-52.
6. Trybek G, Preuss O, Aniko-wlodarczyk M, *et al*. The effect of nicotine on oral health.Balt J Health Phys Act. 2018; 10(2):7-13.
7. Warnakulasuriya S, Johnson NW, vander Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007; 36:575-80.
8. Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med. 2003; 14(1):47-62.
9. Roed-Petersen B, Gupta PC, Pindborg JJ, Singh B. Association between oral leukoplakia and sex, age and tobacco habits. Bull World Health Organ. 1972; 47:13-9.
10. Bánóczy J. Follow-up studies in oral leukoplakia. J Maxillofac Surg. 1977; 5:69-75.
11. Connolly GN, Orleans CT, Kogan M. Use of smokeless tobacco in major-league baseball. N Engl J Med. 1988; 318:1281-S.
12. Chakrabarti A, Chatterjee SS, Rao KL, Zameer MM, Shivaprakash MR, Singhi S, *et al*. Recent experience with fungaemia: change in species distribution and azole resistance. Scand. J. Infect. Dis. 2019; 41:275-284.
13. SoniaSilva, Melyssa Negri. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev. 2012; 36:288-305.