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STUDY OF ORAL MICROFLORA IN SMOKELESS TOBACCO CHEWING SUBJECTS FROM JAMMU REGION

KEY WORDS: oral microflora, dental caries, periodontal disease, smokeless tobacco

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ABSTRACT

Background- Smokeless tobacco chewing deserves a special attention in India, because of its popularity and wide-spread social acceptance.
Aim- To analyze effects of tobacco and betel leaf on the normal microbial flora of the oral cavity in smokeless tobacco chewing subjects.
Methods- Thirty each of smokeless tobacco chewers (case) and non-chewers (control) were randomly selected from the subjects visiting various out patients departments of a dental hospital. Stimulated saliva sample collection was done. Subgingival plaque samples were lifted thereafter. Collected samples were sent to microbiology laboratory for further processing.
Results- Caries experience among chewers, that is those subjects who were affected by caries, was significantly less compared to non-chewers. Among chewers, the most commonly isolated microorganisms were Enterococcus species (93.33%), Lactobacillus species (76.67%), Streptococcus mutans (70.0%), Pseudomonas aeruginosa (50%), Pigmented Prevotella (59.5%), and bacteroides species (46.67%). Non-chewers demonstrated 93.33% isolation of Lactobacillus species, CONS (60%), Bacteroides species (56.67%), Enterococcus species (40%), Peptococcus niger (13.33%).
Conclusion- Greater proportion of caries among non-chewers may be due to greater numbers of Lactobacillus species in this subgroup. Burden of periodontal disease was a little more among chewers compared to non-chewers. These findings suggest lower ability of Gram-negative bacteria to mediate more disease in this subgroup.

INTRODUCTION

Relationship of dynamic equilibrium exists between dental plaque bacteria and the innate host defense system. Use of betel and tobacco may change the normal microflora. The association of microorganisms with dental caries and periodontal disease is well established.[1] The effects of smokeless tobacco on oral microorganisms have been studied in vitro, and it was reported that the sucrose content of tobacco eluants was found to promote the growth of both Streptococcus mutans and Streptococcus sanguinis.[2-4]

Capsicin in chillies has been shown to produce mild changes in connective tissue in wistar rats similar to oral sub mucous fibrosis.[5] The people in rural parts of India mostly use tobacco with lime and keep it into the lower vestibule. Chewing of tobacco and products of betel nut are significantly contributing factors for the oral submucosal fibrosis.[6] In all these cases normal flora of the oral cavity was reduced and they developed submucosal fibrosis which leads to leukoplakia or cancer of the oral cavity.[7-9]

Betel leaves, cardamom, tobacco and clove individually or in different combinations are able to effect on the oral microorganisms. In the region of Jammu, the use of tobacco and betel leaf is quite high. Thus, this study was planned with an objective to analyze effects of tobacco and betel leaf on the normal microbial flora of the oral cavity in smokeless tobacco chewing subjects.

METHODS

The study was conducted at a tertiary care dental teaching hospital of northern India. Subjects were selected by purposive sampling. Thirty each of smokeless tobacco chewers (case) and non-chewers (control) were randomly selected from the subjects visiting various out patients departments of this dental hospital. Those subjects were considered as cases who are currently indulging in the habit of chewing any form of smokeless tobacco like paan tobacco, khaini (tobacco lime), mawa or gutka for a minimum of past one year. Age and sex matched subjects not chewing smokeless tobacco were taken as controls.

Exclusion criteria for this study were subjects having less than 10 teeth in one arch, having a history of major systemic disease or on antibiotics medication within 6 months prior to the study, individuals who had ever smoked tobacco or who had undergone any dental treatment in the past year. Decayed Missing Filled Surface (DMFS) Index, Community Periodontal Index (CPI) Index, Loss of Attachment (LOA) Index were considered to assess the dental caries and periodontal disease status. Duration, frequency and types of smokeless tobacco use was noted down.

Stimulated saliva sample collection was done. Subgingival plaque samples were lifted thereafter. Samples were taken from disto-buccal, buccal, mesio-buccal, mesio-lingual, lingual and disto-lingual sites of each tooth. Collected saliva and subgingival plaque samples were transferred to a test tube containing transport media (thioglycolate) and stored in a refrigerator at lower temperatures till it reached the laboratory for processing.

Collected samples were sent to microbiology laboratory for further processing. The microorganisms were identified based on the colony morphology on the different media. The Streptococcus species were identified on MSA, Lactobacillus species on Rogosa SL agar, Enterococcus on Pfizer selective Enterococcus agar, other aerobic microorganisms on aerobic blood agar and MacConkey agar. The anaerobic microorganisms were identified on the anaerobic blood agar. The detection limit was set at 2000 cfu/ml for saliva and at 8000 cfu/sample for the plaque samples. Subculture, Gram's staining and various biochemical tests such as Catalase test (to differentiate between Staphylococcus and Streptococcus), Coagulase test (to differentiate between Staphylococcus aureus and other species of Staphylococcus), Indole test, Triple Sugar Iron (TSI) agar test, Mannitol motility medium (MMM) test, Urease test and Citrate test (tests for Gram-negative bacilli) were used to confirm the isolates.

Written and informed consent was obtained from study subjects. Permission of ethical committee was obtained from the Institutional Ethics Committee. All the questionnaires were manually checked and edited for completeness and consistency and were then coded for computer entry. After compilation of collected data, analysis was done using Statistical Package for Social Sciences (SPSS), version 21 (IBM, Chicago, USA). The results were expressed using appropriate statistical variables.

RESULTS

The study population comprised of 30 chewers and equal number of non-chewers in the age group of 18-65 years with a mean age of 29 years. Males outnumbered females in this study. Most of cases presented with following: burning sensation in the oral cavity, vesicles and ulcer formation, stiffening of mucosa, reduction in mouth opening, periodontitis, gingivitis and pocket formation, blackening of the teeth, dryness of the mouth, inability to whistle, difficulty in swallowing. Caries experience among chewers, that is those subjects who were affected by caries, was significantly less compared to non-chewers.

In majority of the cases, following were noted on oral examination: white mucosal lining seen in habitual tobacco chewers in the area of tobacco, mucosal alterations, gingival recession, most common area of involvement was the anterior mandibular vestibule followed by posterior vestibule, surface of mucosa of oral cavity appeared white and was granular or wrinkled, periodontal tissue destruction in the immediate area of contact, oral mucosa, appeared grayish white and almost translucent, the area under the tobacco in the oral cavity appeared white, wrinkled or rippled, oral mucosa appeared blanched and slightly opaque, uvula was often reduced in size, with compressed tonsils, fibrosis was seen spreading up to pharynx, in the lip, circular bands of fibrosis around the entire rima oris, and bilateral dark brown hyper pigmentation of the commissure was present.

Among chewers, the most commonly isolated microorganisms were Enterococcus species (93.33%), Lactobacillus species (76.67%), Streptococcus mutans (70.0%), Pseudomonas aeruginosa (50%), Pigmented Prevotella (59.5%), and bacteroides species (46.67%). (Table 1)

Table 1: Organisms isolated from the smokeless tobacco chewers (cases)

Sample	Bacterial growth	Number of patients	Total number of patients	Percentage
Stimulated saliva sample	Enterococcus spp.	28	30	93.33
	CONS [Coagulase negative Staphylococcus spp.]	10	30	33.33
	Lactobacillus spp.	23	30	76.67
	Bacteroides spp.	14	30	46.67
	Streptococcus mutans	21	30	70.00
	Pseudomonas aeruginosa	15	30	50.00
	Klebsiella spp.	12	30	40.00
	Leptotrichia (Anaerobic)	7	30	23.33
	Candida albicans (fungus)	3	30	10.00
	No growth of any micro organisms	6	30	20.00
Subgingival plaque	Veillonella species	6	30	20.00
	Peptococcus niger	7	30	23.33
	Pigmented provetella spp	12	30	40.00
	Fusobacterium spp.	3	30	10.00

Non-chewers demonstrated 93.33% isolation of Lactobacillus species, CONS (60%), Bacteroides species (56.67%), Enterococcus species (40%), Peptococcus niger (13.33%). (Table 2)

Table 2: Organisms isolated from the smokeless tobacco non-chewers (controls)

Sample	Bacterial growth	Number of patients	Total number of patients	Percentage
Stimulated saliva sample	Enterococcus spp.	12	30	40
	Bacteroides spp.	17	30	56.67
	Lactobacillus spp.	28	30	93.33
	CONS [Coagulase negative Staphylococcus spp.]	18	30	60
	Streptococcus mutans	8	30	26.67
	Streptococcus viridans	17	30	56.67
	No growth of any micro organisms	15	30	50
Subgingival plaque	Veillonella species	3	30	10
	Fusobacterium spp.	2	30	6.67
	Peptococcus niger	4	30	13.33
	Diptheroids	1	30	3.33

DISCUSSION

Smokeless tobacco chewing deserves a special attention in India, because of its popularity and wide-spread social acceptance. The present study was carried out to assess the oral microbiota in smoke-less tobacco chewers and non-chewers by microbiological cultures and to compare its relationship with dental caries and periodontal disease status.

The deleterious effects of smokeless tobacco use are perhaps not as well known as those produced by smoking. Smokeless tobacco may increase the risk for oral cancer, induce oral mucosal lesions at the site where tobacco is placed, foster nicotine addiction and dependence and contribute to systemic vascular diseases.[10,11] Fisher et al have indicated that smokeless tobacco may also be an important risk factor for severe active periodontal disease.[12]

In this study, among chewers, the most commonly isolated microorganisms were Enterococcus species (93.33%), Lactobacillus species (76.67%), Streptococcus mutans (70.0%), Pseudomonas aeruginosa (50%), Pigmented Prevotella (59.5%), and bacteroides species (46.67%). Some studies have demonstrated that areca extracts containing arecoline that inhibit growth and attachment of, and protein synthesis in, human cultured periodontal fibroblasts. On the basis of these findings the investigators proposed that areca may be cytotoxic to periodontal fibroblasts and may exacerbate pre-existing periodontal disease as well as impair periodontal reattachment.[12] Some investigators have shown that loss of periodontal attachment and calculus formation is greater in areca chewers Areca- induced lichenoid lesions, mainly on buccal mucosa or tongue, have been reported at sites of quid application. Betel chewers' mucosa is characterized by a brownish red discoloration of the oral mucosa, with tendency for desquamation and peeling. The lesion is usually seen in the area of contact of the betel quid to the buccal mucosa. This lesion is not considered to be potentially malignant, although the condition often co-exist with other mucosal lesions like leukoplakia and submucous fibrosis, which are well known for the malignant change.[12]

Similar study was carried out by Sharad B et al.[3] which showed reduction in oral microbial flora to approximately 56% as compared to control group where as microflora reduction was 50% in the betel leaf chewers.

Chandra and Desai[13,14] have discussed that the probable reasons for low incidence of dental caries in betel and tobacco chewers are because of the alkaline nature of lime and alkaloids, excessive salivary flow, physical action of chewing habit and elimination of caries susceptible pits and fissures by attrition.

Tomar and Winn[15] have reported an increase in caries experience among smokeless tobacco users in United States, which is contradictory to the results of the authors' study. They have attributed the increase in caries level to the high fermentable sugars used in United States that may create an environment conducive to dental caries, but in Indian scenario, the sugars are used to a lesser extent.

CONCLUSION

This study observed that Lactobacillus species, Pigmented Prevotella and Porphyromonas species, and Enterococcus species

were significantly less in chewers than in nonchewers. Greater proportion of caries among non-chewers may be due to greater numbers of Lactobacillus species in this subgroup. Burden of periodontal disease was a little more among chewers compared to non-chewers. These findings suggest lower ability of Gram-negative bacteria to mediate more disease in this subgroup.

REFERENCES

1. Van Houte J. Role of microorganisms in caries etiology. J Dent Res 1994;73:672–681.
2. Lindmeyer RG, Baum RH, Hsu SC, Going RE. In vitro effect of tobacco on the growth of oral cariogenic streptococci. J Am Dent Assoc 1981;103:719–722.
3. Sharad B, Dimple S, Bohra A. Tradition in oral hygiene: Chewing of betel (piper betel leaf) leaves. Current science. 2007 Jan 10;92(1):26-8.
4. Saini S, Saini SR, Katiyar R, Bhalerao DS, Munde A. The use of tobacco and betel leaf and its effect on the normal microbial flora of oral cavity. Pravara Medical Review. 2009;1:47-85.
5. Jha PC, Chakarvarty A, Alok A, Srivastava R, Sanghamesh NC, Khare P. Effect Of Betal Quid And Tobacco On The Normal Flora of Oral Cavity: A Clinical Study. J Clin Den Res Edu. 2014; 3 (7): 49-52.
6. Dockrat I, Shear M. Oral submucous fibrosis in Natal, Fourth Proceedings of the International Academy of oral pathology. New York, 1970, Gordon & Breash.
7. Patel MM, Pandya AM. Relationship of oral cancer with age, sex, site, distribution in habit. Indian J Pathol Microbiol. 2004;47(2);195-197.
8. Pindborg JJ, Is submucous fibrosis a precancerous condition in the oral cavity? Int Dent J, 1972; 22 : 474 -480
9. Shah S, Oral submucous fibrosis : An overview, Marathwada Medical Journal, 2007; 1(1): 19-21 Guggenheimer J. Implications of smokeless tobacco use in athletes. Dent Clin North Am 1991;35:797–808.
10. Robertson PB, Walsh M, Greene J, Ernster V, Grady D, Hauck W. Periodontal effects associated with the use of 55 smokeless tobacco. J Periodontol 1990;61:438–443.
11. Fisher MA, Taylor GW, Tilashalski KR. Smokeless tobacco and severe active periodontal disease, NHANES III. J Dent Res 2005;84:705–710.
12. Ernster VL, Grady DG, Greene JC, Walsh M, Robertson P, Daniels TE et al. Smokeless tobacco use and health effects among baseball players. J Am Med Assoc 1990;264: 218–224.
13. Chandra S, Desai VM. Relationship of betel chewing and dental caries. J Ind Dent Assoc. 1970;42:269–276.
14. Chandra S, Desai VM. Dental caries and betel with tobacco chewing. J Ind Dent Assoc. 1977;49:83–87.
15. Tomar SL, Winn DM. Chewing tobacco use and dental caries among U.S Men. J Am Dent Assoc 1999;130: 1601–1611.