



CHARACTERIZATION OF BACTERIA IN BETEL QUID CHEWERS AND NON-CHEWERS AND THEIR ASSOCIATED ORAL HEALTH STATUS

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ABSTRACT

Aim: To evaluate oral health status, periodontal status, gingival status, oral lesions and bacterial characterization among betel quid chewers and non chewers.

Material and Methods: The data was collected regarding socio-demographic characteristic, oral hygiene status, gingival status, periodontal status, bacterial characterization among betel quid chewers and non chewers. The microbiological examination was carried out to assess the micro flora. The statistical analysis was done by using SPSS version 21(Chicago, USA). The p value ≤ 0.05 was found to be statistically significant.

Results: Aerobic as well as anaerobic bacteria were more characterized among quid chewers as compared to non quid chewers. Significant co-relation was found between oral hygiene status and gingival status, CPI and LOA, oral lesions and bacterial characterization among quid chewers characterization ($r=0.391, 0.932, 572$ respectively p-value=0.000, S) and among non quid chewers significant correlation was found between CPI and LOA scores ($r=0.658$ p-value=0.000, S).

Conclusions: Chewing betel quid has been found in role of deterioration of oral hygiene gingival status periodontal status and also development of oral lesions.

KEYWORDS: betel quid chewers, bacterial characterization, gingival status, non chewers, oral health status

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INTRODUCTION

The oral cavity serves as a gateway to the human body which consisted of several microbial habitats, which includes teeth, gingival sulcus, attached gingiva, tongue, cheek, lip, hard

palate and soft palate. Many surfaces are present in the oral cavity, each coated with a plethora of bacteria, the proverbial bacterial biofilm¹.

Equal proportions of Gram positive cocci, especially Streptococcus sp., and Actinomycetes sp., Yeasts,

protozoa and others are present in the healthy gingival sulcus.² Among these bacteria some are associated with oral diseases such as dental caries and periodontitis, most common bacterial infections reported among humans³.

Human periodontitis is

associated with diverse and complex sub-gingival micro biota encompassing both Gram-positive and Gram-negative bacteria, facultative and anaerobic organisms, and possibly yeasts. Nearly 500 bacterial strains recovered from the sub gingival crevice, particularly well-studied microbial niche^{4,5,6}.

Few diseases are associated with food habits, lifestyle and environmental factors too. It is estimated that about 600 million people chew betel nut world wide.⁷ Betel quid or areca nut has been common in South and South East Asia⁷ Gupta and Warnakulasuria reported that substantial proportion of world's population is engaged in chewing arecanut and this habit is considered as an endemic throughout the Indian sub continent large part of Asia and Melanesia. Along with areca nut, a large variety of ingredients including tobacco may be used constituting a betel quid.⁸ Areca nut is main psycho active substance if tobacco is not used. Nearly three decades ago, a change happened in the betel quid / areca nut use with the advent of "guthka" which is added in betel quid. It consists of tobacco, areca nut and catechu mixed together with several other ingredients believed to be highly addictive, flavored and sweetened, held in the mouth and chewed⁹.

In India, guthka is famous among all socio-economic groups, since it is easily available at affordable cost. It is also have deleterious effects on the oral tissues :dental hard tissues, which includes teeth, periodontium and temporomandibular joint and the soft tissues, which make up the mucosa that lines the oral cavity¹⁰. Also the normal oral microflora changes with the consumption of betel quid. In earlier stages it is due to antimicrobial and synergistic activity of constituents of betel quid i.e. kattha, lime, betel leaf,

betel nut. But in chronic stage, an individual become addictive of betel quid and his normal microflora is replaced by more pathogenic micro-organisms.

As few literatures are available till date to assess the characterization of bacteria in betel quid chewers and non chewers and no such clinical microbiological study has been conducted in Rajasthan. Keeping these points in view the study has been conducted in Jodhpur city with following aim and objectives: (1) to evaluate the effect of betel quid chewing on oral hygiene status; (2) to evaluate the effect of betel quid chewing on gingival status; (3) to evaluate the effect of betel quid chewing on periodontal status; (4) to assess the association between characterization of bacteria among betel quid chewers and non chewers.

MATERIAL AND METHODS

A cross-sectional study was carried out in Jodhpur city among betel quid chewers and non chewers .A total of 80 patients of both the genders (40 betel quid chewers and 40 non- chewers) aged between 15 to 60 years were randomly selected from the outpatients attending the associated group of hospitals of Dr .S.N.Medical Collage Jodhpur.

The Inclusion criteria was: subjects with at least 20 permanent teeth including all the index teeth; in case of chewers - presence of chewing habit for a minimum of 6 months duration and consuming at least 4-5 quids per day.

The Exclusion criteria was: patients who had undertaken periodontal therapy and those who had been on antibiotics in past 3 months; patients with systemic illness; pregnant women.

Ethical clearance was obtained from the Ethical Committee of Vyas Dental College and Hospital Jodhpur and

also permission was taken from Principal of Dr.S.N.Medical College and Department of Microbiology,Umaid Hospital to perform the study. An informed consent was obtained from the subjects prior to the study.

The data collection was done by using preformed performa which included the socio-demographic details, details regarding the adverse oral habits,clinical examination which included the indices(OHI-S, Loe and Silness gingival indexand CPI& LOA (W.H.O.modified) and microbiological examination.

Microbiological examination: Study participants rinsed their mouth with 10 ml of sterile water for 15 sec then samples were taken by scrubbing the either side of the cheek with sterile cotton swab, two swabs were taken, one was used for culture & another for direct microscopy and smear examination. With first swab direct microscopic examination was done by doing wet mount, 10%KOH examination,Gram's smear and if any special stain like ZiehlNeelson stain or Albert stain was done for Mycobacterium and Corynebacterium respectively.

By doing wet microscopy predominant flora and type of epithelial cells were examined.

By Gram's smear again type of flora was evaluated and culture was done accordingly.

For culture, samples were transported to microbiology laboratory in thioglycolatebroth,a transport media and was used for culture on blood agar, maconky agar and Sabouraud's dextrose agar for identification of pathogenic bacteria or fungi respectively and semi quantitative culture was done.

Steps in Identification of bacteria includes: Morphology of bacteria, Growth on culture media,

Staining, Biochemical tests. Also the identification of any fungal growth can be identified by following step: Direct microscopy, Culture.

RESULTS

Graph 1-4, shows the socio demographic characteristics and their bacterial characterization among betel quid chewers which revealed that among gender, males were having more contamination with aerobic 74.5% and anaerobic bacteria 1.8% as compared to females, while keeping living condition in consideration urban people were showing more contamination with aerobic 52.7% and anaerobic bacteria 1.8% and fungal 5.5% as compared to rural people and periurban. Our study shows that with increase in education and income level the incidence of bacterial contamination was also increasing.

Table 1 shows that the study subjects who were having poor oral hygiene were showing aerobic 14.5% and anaerobic bacteria 1.8% whereas those who were having fair oral hygiene are showing aerobic 67.3% as compared to good oral hygiene study subjects.

When we compared the gingival status, then the study subjects who were having mild gingival inflammation were showing aerobic 56.4%, anaerobic bacteria 1.8% and 3.6% fungal whereas those who were having severe gingival inflammation are showing aerobic 21.8% and 3.6% fungal contamination as compared to study subjects having moderate gingival status. Increasing CPI scores were directly proportional to the bacterial characterization that is subjects who were having calculus and pocket depth of 4-5mm were showing more aerobic bacteria (67.3%) and fungal (7.3%) as compared to their counterparts. Loss of Attachment was more among

subjects who were having 0-3mm and 4-5mm pocket depth as compared to others.

Table 2 indicated that among oral lesions, leukoplakia (18.2%) and ulceration (16.4%) were more commonly associated with aerobic bacteria as compared to lichen planus and malignant tumors (10.9%). Study subjects showing candidiasis were showing more fungal contamination (10.9%) as compared to aerobic bacteria. Interestingly it was found that the most common location of oral lesions recorded was buccal mucosa.

One way ANOVA was applied to test the difference between oral hygiene status, gingival status, periodontal status, oral lesions and bacterial characterization among betel quid chewers and non chewers which was found to be statistically significant ($p = 0.000$) (Table 3). This shows that quid chewers were more affected from dental diseases as compared to non chewers.

A significant correlation was found between oral hygiene status and gingival status, CPI scores and LOA scores and also in between oral lesions and bacterial characterization ($r=0.391, 0.932, 572$ respectively $p\text{-value}=0.000, S$) among quid chewers in Table 4.

Table 5 shows, a significant correlation was found between CPI scores and LOA scores ($r=0.658$ $p\text{-value}=0.000, S$) but no other correlation was found between other variables among non chewers.

Table 6 shows that the study subjects who were having good oral hygiene were showing aerobic 77.8% bacteria as compared to others and the study subjects who were having mild gingival inflammation were showing aerobic 86.7% whereas those who were having moderate gingival inflammation were showing aerobic 13.3%. CPI scores

were directly proportional to the bacterial characterization that is subjects who were having calculus were showing more aerobic bacteria (64.4%) as compared to their counterparts. Loss of attachment was more among subjects who were having 0-3mm pocket depth as compared to others.

In Table 7, characterization of bacteria among betel quid chewers and non chewers was compared, it was found that there was significant presence of streptococcus species among non quid chewers as compared to quid chewers, among them all the other bacteria were also significantly characterized whereas E. coli, Citrobacter and fusobacterium were showing no association among the groups.

One way ANOVA was applied to test the difference between bacterial characterization among betel quid chewers and non chewers which was found to be statistically significant ($p = 0.000$). This shows that quid chewers were more affected from bacteria as compared to non chewers in Table 8.

DISCUSSION

Habitual eating of betel quid causes discolouration of teeth, dental caries, worn out dentition and periodontal diseases. Therefore we conducted a study comprised of 80 subjects attending outpatient Dr .S.N. Medical College Jodhpur. In this study, prevalence of oral hygiene status, gingival disease, periodontal disease, oral mucosal condition and bacterial characterization among betel quid chewers and non betel quid chewers was evaluated.

Normal oral cavity consists of a mixture of organisms which includes bacteria, fungi, protozoa and occasionally viruses. Some of the organisms are oral

commensals and are called resident flora. Bacteria are most predominant among all the microorganisms present in the mouth which includes both aerobic as well as anaerobic organisms.¹¹ The anaerobic flora shows predominance of Lactobacilli, Leptotrichiabuccalis and Veillonella. Aerobic flora normally consists of - Streptococcus viridans, Coagulase Negative Staphylococci (CONS), Diptheroids and Neisseria catarrhalis. Candida albicans is the most common fungus isolated from mouth. The count of normal flora is more than one lakh bacteria/ml of saliva (CFU (colony forming unit)).

Reduced microbial flora was shown in the present study, which includes Streptococcus viridans, some microbial flora showed environmental bacteria like Pseudomonas aeruginosa, Klebsiella pneumonia, Klebsiellaoxytoca, Moraxcellacatarrhalis, Micrococci and Candida albicans were grown. Anaerobic bacteria were least seen in our cases. In our study males had more aerobic contamination than females. The reason for such finding is that females maintain good oral hygiene for aesthetic purpose as compared to males. Most of the reports suggest that there is no sex predilection.

High incidence of disease was associated with the quid usage.¹² Some studies have found that areca extracts containing arecoline that inhibits growth and attachment as well as protein synthesis in human cultured periodontal fibroblasts. Therefore, these findings make the investigators to propose that quid as cytotoxic to periodontal fibroblasts and may also exacerbate pre-existing periodontal disease as well as impair periodontal reattachment.¹³ Betel quid is nowadays an alarming issue, especially in socially disadvantaged societies. Lack of knowledge, awareness,

poverty as well as low income, are the most common reasons that one adapts this habit of chewing.

The highly significant relation was found between variables (oral hygiene status, gingival disease, periodontal disease, oral mucosal condition and bacterial characterization) and betel quid chewers in this Choudhury et al¹⁴ reported that betel quid chewing leads to poorer periodontal health. Ling et al¹⁵ showed 42.6% bleeding on probing in betel quid chewers and greater pocket depth. Mehta et al¹⁶ reported that teeth become dark brown from chewing betel leaves because of deposits of lime on teeth.

Some researchers have shown that periodontal loss of attachment and calculus formation increased among quid chewers. Betel quid chewers mucosa is characterized by a brownish red discoloration of oral mucosa, with propensity for desquamation and peeling. The lesion is commonly seen in relation to the area of contact of the betel quid that is buccalmucosa. Although the condition often coexists with other mucosal lesions like leukoplakia and submucous fibrosis, which are well known for the malignant change but this lesion is not considered to be potentially malignant.¹³ Oral sub mucous fibrosis is a chronic condition and occasionally vesicles appear. Patients with severe cases face difficulties while chewing, swallowing and speaking and also many exhibit atrophy of the lingual papillae.¹⁷

CONCLUSIONS

The study revealed that betel quid chewers had poor oral hygiene, increased gingival inflammation and increased loss of attachment compared to non chewers. Also it is observed that chronic betel quid chewing

cause oral lesions which can be the pre-neoplastic lesions of oral cavity and subsequently malignancy. An anti-betel quid chewing programs should be emphasized in public prevention. Education and regular screening should be done to prevent oral cancer. As betel quid chewing is rooted in Indian tradition an anthropological study should be indicated for designing appropriate education campaigns.

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