MICROBIOLOGICAL ASSESSMENT IN TOBACCO POUCH KERATOSIS

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Abstract

Background: The current research was conducted with an aim to evaluate the association of oral microbiome with potentially malignant disorders(PMDs) because tobacco usage in any form can cause dysbiosis by altering the normal microbiome. Thus, our definitive knowledge of the oral commensal bacteria can definitely be used as a potential adjunct to early diagnosis and management of PMDs.
Aim: To assess the microbiological pattern in tobacco pouch keratosis lesions among migrant construction workers in Chennai.
Objectives:1.To evaluate the medicinal potential of Bromelain against tobacco pouch keratotic lesions
2.To examine the potency of plant extract by determining MIC (Minimum Inhibitory Concentration)
Methods: Here, we used standard microbiological techniques to analyze the microbiota from labial and buccal mucosa of 70 smokeless tobacco users to assess microbial composition and effects of smokeless tobacco. In vitro experimental study was carried out to evaluate the antimicrobial effects of bromelain against MDR microbes.80% methanolic extraction was employed.MIC were measured using micro dilution as per CLSI protocols.
Results: The distribution of microbial isolates from smokers is shown in Table 1. Streptococcus mutans was the most prevalent bacterial isolate followed by Candida species among smokers. The potency of plant extract was examined by determining MIC.MDR isolates including C.albicans and S.mutans were significantly inhibited by plant extract. Test extracts showed promising antibacterial potential against S.mutans with low MIC values ranging between 0.019mg/ml and 10mg/ml.
Conclusion: The ecological shift to dysbiosis is a significant finding in oral carcinogenesis.The Bromelain extract has shown appreciable antimicrobial activities against MDR pathogens. Further investigation on a larger group of altered microbiomes can help in appropriate treatment and better prognosis by establishing relationship of altered microbiome.

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1. Introduction

Oral cancer is the fifth most common cancer worldwide. Tobacco use leads to major deleterious habits on the oral mucosa[1-1,2]. A causal association has been established between tobacco,betel quid chewing habits and oral cancer[3].The prevalence of tobacco use among Indian adults is 35%[4-5].In India, beedi and paan are the most popular forms of tobacco consumption[6]. Paan masala, gutkha and mawa are the most popular and highly addictive preparations among dry tobacco areca nut[7-7,8].

Oral cavity is prone to innumerable changes because of environmental and lifestyle related factors in addition to advancing age[1,2,9-10].Infections, local trauma or irritation, systemic diseases and excessive consumption of tobacco, betel liquid and alcohol can contribute to oral mucosal lesions[11].Oral mucosal lesions like leukoplakia, erythroplakia, submucous fibrosis are considered as precancerous lesions associated with the use of tobacco[12].It is thereby important to reduce the incidence of oral cancers by focusing on identification, secondary prevention strategy and management of pre cancerous lesions[13].

Potential health risks are associated with diverse microbial elements known to be present in smokeless tobacco products(STPs)[14].Common consumption practices include chewing, sniffing or placing the product between the gums and cheeks where it releases nicotine and other substances[15].

Ananas comosus L., also known as, pineapple belongs to the family of Bromeliaceae. The selected plant in the study have remained as an integral part of traditional medicine Pineapple is native to Thailand, China, Brazil and India. Pineapples comprise phytochemicals that are considered as essential bioactive compounds for health[14,16-17].Bromelain is a cysteine protease found in pineapples[18]. It is employed in various therapeutic areas because of its anti-inflammatory and anti-cancer activities[19-20].

Tamil Nadu is a highly urbanized state in India and interstate migrant laborers working here constitute a marginalized community. It is generally perceived that consumption of tobacco and alcohol among the workers is high. Very few studies have been undertaken to address the type or quantities of microorganisms that have been associated with the human diseases like oral cancer, pulmonary inflammation,chronic bronchitis and other opportunistic infections.Our team has extensive knowledge and research experience that has translate into high quality publications [21–30].

Therefore, the goal of this study was to conduct a microbiological survey of tobacco pouch keratosis among the workers by investigating the antimicrobial activity of pineapple plant variety which are potentially important to public health for the prevention of infectious diseases.

2. Methodology

This study was conducted in different construction sites through oral health screening camps across Chennai and Thiruvallur. The ethical approval was obtained from Institutional Human Ethics Committee, Saveetha Dental College and Hospitals as the research involved reviewing of human subjects. Convenient sampling is done.

Study Procedure

The undergraduates and postgraduates of department of Public Health Dentistry were trained for a duration of 2 months to take data from the study sample.First,the purpose of the study was explained to the study participants and informed consent was obtained from them.A face-to-face interview was conducted and their oral cavity was clinically examined using mouth mirror and explorer to identify tobacco related oral lesions. All the oral lesions were clinically diagnosed as per WHO criteria.Information regarding the demographic characteristics was collected using a questionnaire formatted in Hindi and local language Tamil.The validity and reliability of the questionnaire were pretested.

Inclusive criteria:

Chewing tobacco for atleast 5 years

Not taking any medicine (antibiotic/fungal/corticosteroid) for 6 months

With no systemic conditions

Not wearing any kind of prosthesis

Collection and isolation of bacteria:

Exfoliated oral cells were collected form the upper and lower lips, buccal mucosa and the surface of the gingiva using saline moistened cotton swabs.During this study,70 subjects indulged in smoking(smoke or smokeless forms) were recruited.The swab sticks were transferred within 1 hour to the Microbiology Laboratory of Saveetha Dental College and Hospital for analysis

Extract preparation

Pineapple was washed thoroughly under running tap water,rinsed in distilled water and air dried at room temperature.The fruit was then reduced to appropriate size.The pineapple was weighed by sensitive digital weighing balance and a total of 92.94g of cut pineapple was macerated with 80% methanol for three days at room temperature(Fig 1 and Fig 2).The extraction process was facilitated by occasional shaking.After three days,the resultant liquid was filtered using Whatman filter paper No.3.

For aerobic bacterial cultures

The specimens were inoculated directly onto Mac Conkey,10% sheep blood agar media,chocolate bacitracin blood agar,gentamycin blood

agar,mannitol salt agar and Thayer Martin(contain vancomycin to inhibit gram positive bacteria and colistin to inhibit gram negative bacteria).All plates were incubated at 37 degree Celsius for 24 h in the aerobic and CO2 incubators depending on the media inoculated.If there was no bacterial growth,the medium was re incubated for 24h more before it was released as negative.

For anaerobic bacterial cultures

The specimens were inoculated on sheep blood agar,neomycin anaerobic blood agar,bacteriode bile esculineagar,brain heart infusion agar and tube of thioglycolate broth as soon as possible. The plates were incubate at 35 degree Celsius for 48 h.If direct cultures were negative after 48 h incubation,we repeated subcultures from thioglycollate. If no growth was detected after 96 h, the original plates were reported to be negative.

Minimum Inhibitory Concentration (MIC) Determination

Antibacterial activities of the extracts were first screened by agar well diffusion method.The MIC testing was performed for all the plant extracts that were judged as active (inhibition zone > 7mm) against atleast on test organism by agar well diffusion method.The MIC values were determined by the MHB microbroth dilution method using 96 well plates.The lowest concentration of the extract with clear suspension was considered as the MIC Values(Fig 3 and Fig 4).

3. Results

Table 1:MIC of the bacterial strains used in the study

Sr.No	Concentration	Candida albicans	Candida tropicalis	Candida glabrata	Streptococcus mutans
1	10 mg/ml	MIC	Growth	Growth	MIC
2	5 mg/ml	Growth	Growth	Growth	MIC
3	2.5 mg/ml	Growth	Growth	Growth	MIC
4	1.25 mg/ml	Growth	Growth	Growth	MIC
5	0.625 mg/ml	Growth	Growth	Growth	MIC
6	0.312 mg/ml	Growth	Growth	Growth	MIC
7	0.156 mg/ml	Growth	Growth	Growth	MIC
8	0.078 mg/ml	Growth	Growth	Growth	MIC

9	0.039 mg/ml	Growth	Growth	Growth	MIC
10	0.019 mg/ml	Growth	Growth	Growth	MIC

Fig 1:Digital Weighing Balance

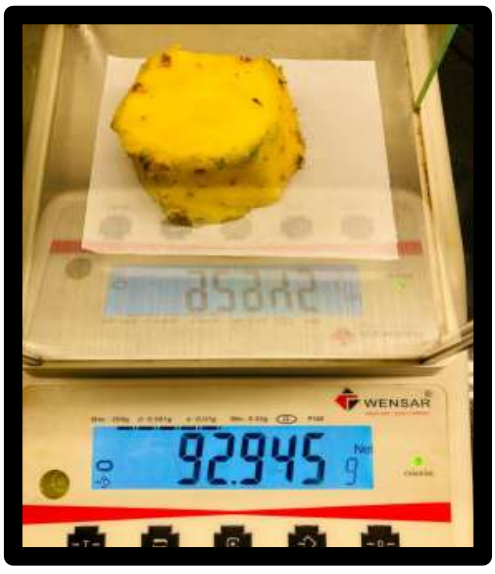

Fig 2:Methanolic extract of pineapple

Fig 3: Growth of Candida species

Fig 4: Inhibition of S.mutans

4. Discussion

Animal studies have shown Pan masala and gutkha to be carcinogenic. Exfoliated buccal mucosal cells of pan masala chewers have manifested increased cytogenetic damage .These genotoxic effects are most likely caused by tobacco and areca nut specific ROS and nitroamines.

A strong antibacterial activity (MIC =49ug/mL) was observed for the extracts from *Curculigoorchioides*and *Cinnamomum camphora*against S.pyogenes,followed by Curcuma longa extract against E.faecalis (MIC=98ug/mL)[31].Our results are in agreement with these findings where bromelain extract exhibited a strong antibacterial activity against S.mutans both at high concentration of 10mg/mL and 0.019 mg/mL.

According to Nasreen et al,there was a more significant increase in the biofilm formation of smoker isolates at 1,4,8,16 and 32 mg/ml compared to biofilm at the xero nicotine concentration[31,32]. Thus,the study indicated that S.mutans isolates from the smokers are more influenced by high nicotine concentrations than non smokers. However,our study did not incorporate the collection of swab samples from non smokers. Therefore,study of larger cohorts who did not indulge in tobacco would be important for a better understanding of the study.

Numerous tudies have revealed that S.mutans and C.albicans are found together in dental plaques from toddlers with ECC.Consequently,theuse of nicotine products increases the growth of S.mutans and inceases the risk for dental decay among tobacco users[31–33]. In a study conducted by Shiyu Liu et al,biofilmformation,bacterial and fungal cell members in dual species biofilm(S.mutans and C.albicans) increased in the presence of nicotine.Hence,it has been reported that S.mutans and C.albicans are putative pathogens for dental caries.The intensification of the synergistic relationship by nicotine may contribute to caries development in smokers[34].

In a study conducted by Jing Han et al,the most common bacteria identified among the STPs were members of the genus Bacillus.The species of Bacillus,B.licheniformis and B.pumilus are potential causes of pulmonary inflammation and opportunistic infections[35].These findings are in contrast to our study where the study samples yielded growth of C.albicans,C.tropicalis,C.glabrata and S.mutans.

Strengths

To the best of our knowledge,this study is the first to investigate the relationship between oral

microbiome and tobacco pouch keratotic lesions. Alterations in several microbial colonies were found to be associated with precancerous lesions.The study supports the hypothesis that oral microbiota may play a pivotal role in oral cancer etiology.

Limitations

The study presented here has certain limitations. Firstly, the sample size of the study was relatively small and limited to a set of individuals working in construction sites in Chennai. The study of larger cohorts that do not practice the use of tobacco would be important for a better understanding of the study. The study of individuals who have quit using tobacco would be important to reveal how long a normal microbiome persists and how long it takes to recover after the use of tobacco.

5. Conclusion

Smokers had a diverse microbial colonization than nonsmokers. Our study suggests that smoking alters oral mucosal colonization in favour of periodontal pathogens.

In summary, we found evidence that specific bacterial pathogens may play a significant role in formation of precancerous.However,larger studies are needed to confirm our findings,particularly among smokers and nonsmokers to clarify causal relationships.This study provides an insight into smokeless tobacco product associated bacteriome and their potential in the progression of oral diseases. Besides,it will assist the government organizations for better management and cessation of tobacco use.Community outreach programmes should promote awareness for the public on the health implication of smoking and poor oral practice.

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