

Effect of Tobacco Smoking on Oral Microbial Flora and the Relationship with Oral Health in Calabar, Nigeria

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Cigarette smoking is a public health problem. It decreases the commensal population of normal flora in the oral cavity leading to an increase of pathogenic microbes. It causes oral cancer, periodontitis, colour change on the teeth, halitosis and other health implications. The study was designed to determine the changes caused by tobacco smoking on the microbial profile and oral health conditions of cigarette smokers. One hundred and twenty subjects comprising 60 tobacco smokers and 60 non smokers were enrolled for the study. Oral swabs were collected from the oral cavity of the subjects using sterile swab sticks under standard aseptic methods. The specimens were subjected to microscopy and culture. Organisms were identified using standard microbiological techniques. Higher rates of microbes 86.7% were recovered from the oral cavity of smokers than non smokers 33.3%. There was a statistically significant effect of tobacco smoke on the oral flora of smokers ($\chi^2 = 299.0$, $P = 0.0002$). *Staphylococcus aureus* 13(25.0%) and *Klebsiella pneumoniae* 10(19.2%) were more prevalent among smokers, while *Klebsiella pneumoniae* 4(20.0%) and *Pseudomonas aeruginosa* 4(20.0%) were the most prevalent bacterial isolates among the control subjects. Smokers had a diverse microbial colonization than non smokers. Smoking may have altered bacterial acquisition and oral mucosal colonization in favor of periodontal pathogens. The campaign against smoking should therefore be intensified as this may help to improve the oral health conditions of smokers.

Key words: Tobacco smoking, Microbial flora, oral health

Introduction

Cigarette smoking is a public health issue. It is the major cause of oral cancer, periodontitis, colour change on the teeth, halitosis and other health implications. It brings about a drastic decrease in the commensal population of normal flora in the oral cavity leading to an increase of pathogenic microbes [1]. Cigarette smoking could enhance microbial colonization by biofilm formation on oral epithelial cells. This may impair host immune responses against pathogens and also disrupt effective nasal mucociliary clearance [2-3].

A number of studies have reported that smoking increases the probability of extensive disease development [4-5].

Other oral lesions caused by cigarette smoking includes: periodontal disease, keratotic patches, nicotinic stomatitis, palate erosion, tooth loss and caries, oral cancer, smokers melanosis etc[6].

There are over 1 billion adult smokers worldwide [7]. Tobacco smoking causes about 20% of deaths in the United States every year [8].

The mouth is an ecological niche of many microbial communities with important implications to human health and diseases. The oral cavity is made up of; the lips and its inner lining, buccal mucosa, teeth, gums, the

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front two-thirds of the tongue, and the hard palate [9-10].

Oral health reflects the well being of an individual, thus maintaining oral hygiene is important [11]. The indigenous oral flora plays an important role in health and disease of the host immune system. They also provide resistance by competing for colonization sites of pathogenic microorganisms [12]. The maintenance of proper oral hygiene can reduce oral microbial load. This results in the control of oral diseases [11].

Microorganisms which cannot be avoided in our immediate environment are sometimes introduced into the oral cavity by the use of substance that have been contaminated due to poor handling such as; cigarette, contaminated food, water, toothbrush and so on [13].

Some pathogenic microbes such as; *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus species*, *Escherichiacoli*, *Pseudomonas aeruginosa*, *Candida species*, *Entamoeba gingivalis* and *Porphyromonas gingivalis* may be present in the oral cavity due to poor oral hygiene. These microbes can cause diseases and disorders such as; gingivitis, pharyngitis, stomatitis, tonsillitis, sinusitis, dental decay, oral thrush, halitosis, periodontitis, oral cancer and so on [14].

The number of smokers in our Nation is on the increase despite the warnings against cancer and other related diseases but little is known about the microbial profile of smokers' oral cavity in our locality. The care for the oral cavity is not taken seriously due to poor oral hygiene measures among smokers and non-smokers. This study will shed more light on the possible health implication of smoking in relation to oral health among our subjects. The aims and objectives of this study were to: Determine the microbial profile in the oral cavity of cigarette smokers and non-smokers, compare the bacterial acquisition and colonization in smokers to that of non-smoker and to check if smokers are more prone to oral cavity diseases.

Materials and methods

Study Area

The cross sectional study was carried out in Calabar municipality, Nigeria. She is bounded by Odukpani Local Government Area, the great Kwa River, Calabar River and Calabar South Local Government Area in the North, East and South. Calabar has an area of 142km² and a population of 179,392 [15].

Sample collection

One hundred and twenty subjects were enrolled for the study after signing informed consent form. They comprised 60 cigarette smokers and 60 non smokers. A structured questionnaire was administered to participants for data collection on demography, history of oral hygiene, oral infections, smoking habits and so on. Ethical approval was obtained from the Ethical Research Committee, University of Calabar Teaching Hospital.

Convenient sampling method was used. Oral swabs were obtained from all the subjects using sterile swab sticks. The swab sticks were transferred within 1 hour to the Microbiology Laboratory in The University of Calabar Teaching Hospital (UCTH) for analysis.

Inclusion and exclusion criteria

Subjects aged 18 years and above who had been smoking tobacco products regularly for at least one year were enrolled as test subjects while those aged 18 years and above who had never smoked were enrolled as controls.

Exclusion criteria

Females were not included in the study as this habit is not acceptable among women in our locality. We thought it would be difficult to recruit females who admit they were smoking.

Processing of Sample

The swab sticks were aseptically cut from the handle and placed in 5mls of peptone water broth. The broth was incubated at 37°C for 2 hours and used as stock.

Culture

A loopful of the stock was picked using a sterile calibrated wire loop that holds 0.002ml and inoculated on Blood agar, Cystein Lactose Electrolyte Deficient agar (CLED) and Sabouraud dextrose agar. The CLED were incubated aerobically at 37°C for 18-24 hours. Blood agar plates were incubated in a CO₂ jar at 37°C

for 18-24 hours. Sabouraud dextrose agar was incubated at room temperature and at 37°C aerobically for 7 to 14 days.

Identification of Isolates

Isolates were identified macroscopically, microscopically and biochemically. Isolates were examined with unaided eye for colour, size, consistency, shapes, odour and so on. Isolates were examined microscopically by Gram staining technique and Lactophenol cotton blue mounts. Physiological/Biochemical tests carried out include: Coagulase test, Catalase test, Citrate utilization, Indole test, Oxidase test, Urease test, inoculation on Kligler iron agar (KIA) and germ tube test for *Candida* species.

Results

Figure 1 shows the age distribution of the study population. The mean age among the subjects was 26.9±3.4 years, with minimum age 18.0 years and maximum age 36.0 years. There was no statistically significant relationship between age and smoking ($\chi^2 = 18.6, P = 0.23$).

The distribution of microbial isolates from smokers and non smokers is shown in Fig. 2. More microbes were recovered from the oral cavity of smokers than non smokers. *Staphylococcus aureus* 13(25.0%) was the most prevalent bacterial isolate followed by *Klebsiella* species 10(19.2%) among smokers while *Pseudomonas aeruginosa* 4(20.0%) and *Klebsiella* species 4(20.0%) were the most prevalent isolates among non smokers. *Serratia marcescens* and *Citrobacter freundii* were not associated with non smokers.

Table 1 shows the relationship between oral cavity disease and oral microbial flora among smokers. A total of 52 microbial isolates was associated with the smokers. Tooth decay 19(36.5%) was the oral cavity disorder among smokers associated with the highest number of isolates, followed by halitosis 18(34.6%) and mouth ulcer 7(13.4%). *Staphylococcus aureus* was the most common isolate 13(25.0%) with its peak in tooth decay 5(38.4%) followed by *Klebsiella pneumonia* 10(19.2%) with its peak in tooth decay 4(40.0%). Most of the *Candida* species were recovered from subjects with halitosis 5(71.4%). There was a statistically significant association between oral cavity conditions and microbial isolates among smokers ($\chi^2 = 299.0, P =$

0.0002).

Table 2 shows the relationship between microbial flora and oral cavity conditions among non smokers. A total of 33.3% infection rates were associated with non smokers. Halitosis 17(85.0%) was the most common oral cavity condition among non smokers, followed by tooth decay 2(10.0%). There was no statistically significant association between oral cavity conditions and microbial flora among non smokers ($\chi^2 = 127.0, p = 0.50$).

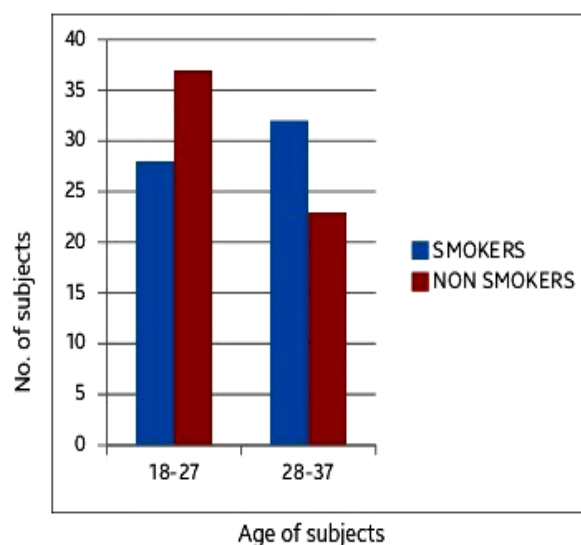


Fig.1 Age distribution of subjects

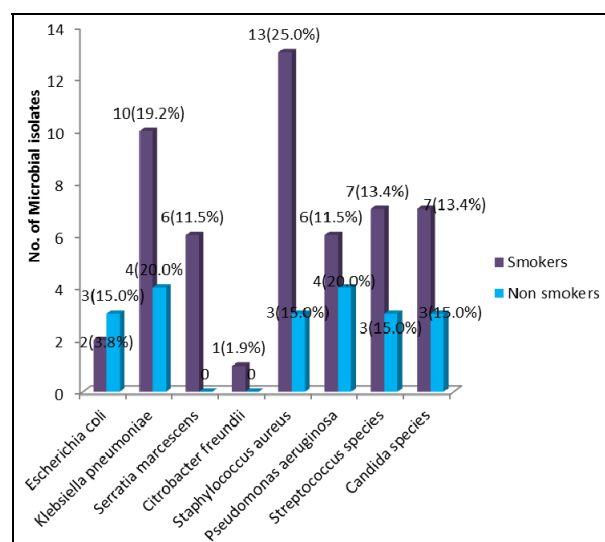


Fig.2 The distribution of microbial isolates among subjects

Table 1 The relationship between oral microbial flora and oral cavity disease among smokers

Microbial isolates	Oral cavity diseases No (%) of isolates						Total
	Halitosis	Mouth ulcer	Tooth decay	Blackening	Blackening of teeth & Halitosis	Tooth decay & Halitosis	
<i>Escherichia coli</i>	1(50.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	2(3.8)
<i>Klebsiella pneumoniae</i>	2(20.0)	2(20.0)	4(40.0)	2(20.0)	0(0.0)	0(0.0)	10(19.2)
<i>Serratia marcescens</i>	1(16.7)	2(33.3)	1(16.7)	2(16.7)	0(0.0)	0(0.0)	6(11.5)
<i>Citrobacter species</i>	1(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.9)
<i>Staphylococcus aureus</i>	4(30.8)	2(15.4)	5(38.4)	2(15.4)	0(0.0)	0(0.0)	13(25.0)
<i>Pseudomonas aeruginosa</i>	2(33.3)	1(16.7)	2(33.3)	0(0.0)	1(16.7)	0(0.0)	6(11.5)
<i>Streptococcus species</i>	2(28.6)	0(0.0)	5(71.4)	0(0.0)	0(0.0)	0(0.0)	7(13.4)
<i>Candida species</i>	5(71.4)	0(0.0)	2(28.6)	0(0.0)	0(0.0)	0(0.0)	7(13.4)
Total	18(34.6)	7(13.4)	19(36.5)	6(11.5)	2(3.8)	0(0.0)	52(86.7)

Table 2 The relationship between oral microbial flora and oral cavity disease among non-smokers

Microbial isolates	Oral cavity diseases No (%) of isolates						Total
	Halitosis	Mouth ulcer	Tooth decay	Blackening	Blackening of teeth & Halitosis	Tooth decay & Halitosis	
<i>Escherichia coli</i>	3(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(15.0)
<i>Klebsiella pneumonia</i>	4(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(20.0)
<i>Serratia marcescens</i>	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
<i>Citrobacter species</i>	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
<i>Staphylococcus aureus</i>	2(66.7)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(15.0)
<i>Pseudomonas aeruginosa</i>	3(75.0)	0(0.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	4(20.0)
<i>Streptococcus species</i>	2(66.7)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	3(15.0)
<i>Candida species</i>	3(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(15.0)
Total	17(85.0)	1(5.0)	2(10.0)	0(0.0)	0(0.0)	0(0.0)	20(33.3)

Discussion and conclusion

In this study higher rates of microbe were recovered from the oral cavity of smokers 86.7% than non smokers 33.3%. This finding agrees with the report of Kubota *et al* [16] who found that the detection rate of periodontopathic bacteria were higher in smokers. However, it is different from the report of Sreedevi *et al* [17] who reported that there was no difference in the periodontal microbiota status between smokers and non smokers. This difference may have resulted because of the types of sample obtained for analysis. In this study, oral swabs were obtained for culture while Sreedevi *et al* [17] analyzed periodontal plaques using BANA tests.

Females were not included in the study as this habit is not acceptable among women in our locality. Sreedevi *et al* [17] who conducted a similar study in Bangalore, India also excluded female subjects from their study because of the same reason and to avoid potential hormone-induced microcirculatory changes.

In this study, *Staphylococcus aureus* (81.3%) and *Klebsiella* species (71.4%) were the most prevalent bacteria among smokers while *Klebsiella* species

4(28.6%) and *Pseudomonas aeruginosa* (40.0%) were more prevalent among non smokers. Our findings is slightly different from the report of Wetzel *et al* [14] who isolated *Streptococcus mutans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the most prevalent among smokers. The slight variation of microbial flora among our subjects may be due to variation in oral hygiene habits. Although we did not investigate the source of the organisms in our study, Sapkota and colleagues [18] in the USA recovered these organisms from the cigarettes. The isolates in this study are potential pathogens or opportunistic pathogens of the upper and lower respiratory tract as well as the oral cavity.

Comparing the oral microflora of smokers with non smokers we observed a divergent of oral microbiota comprising a well-defined transition from gram-negative to gram-positive dominated community in non smokers. *Staphylococcus aureus* and gram-negative organisms including; *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens* were more prevalent in smokers. *Serratia marcescens* and *Citrobacter* species were not associated with non smokers. *Escherichia coli*, *Streptococcus* species and *Candida* species were well

distributed in both groups and are probable members of the core microflora of the oral cavity.

Tobacco use is associated with a range of changes to the oral mucous membranes. Though the effect of exposure to tobacco smoking was not investigated among our subjects, smokers had higher occurrence of oral infections and abnormalities 52(86.7%) than non smokers 20(33.3%). Tooth decay 19(36.5%) was the most occurring oral disease among smokers followed by Halitosis (bad breath) 18(34.6%). There was a statistically significant association between oral cavity disease and microbial isolates among smokers. This shows that smoking affects oral microbial acquisition and the oral cavity diseases.

Mouth ulceration and blackening of teeth were more prevalent among smokers than non smokers. The ulceration among smokers could have been due to the proliferation and invasion of the oral mucosa by organisms introduced through cigarette sticks or the reduction of normal oral flora by the smoke thus making room for opportunists to thrive.

Smokers 19(36.5%) suffered more tooth decay than non smokers 2(10.0%). This could be due to the fact that they indulged in habits such as chewing gums and licking of sweets as mouth fresheners after smoking which made them susceptible to tooth decay.

Conclusion

Smokers had a diverse microbial colonization than non smokers. Our study suggests that smoking alters bacterial acquisition and oral mucosal colonization in favor of periodontal pathogens. Awareness should be created for the public on the health implication of smoking and poor oral practice.

Limitations of the study

The limitations of the study include the inability to obtain multiple samples from subjects due to the subject's inaccessibility. This could have given a picture of transient oral flora and the permanent flora of the subjects.

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