A comparative study of microbial floral of toothbrush used by smokers and non smokers in Ekpoma.

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ABSTRACT
The aim of this study was to assess the occurrence and intensity of microbial floral present in the normal oral cavity of smokers and non-smokers in Ekpoma and environs. In order to understand the temporal effects of smoking on oral bacterial colonization, a cross-sectional survey of 200 individuals in Ekpoma and environs was recruited for the study. Amongst the 100(50%) subject smokers only two 4(4%) were females and 96(96%) were males while, out of the fifty 100(50%) of non-smokers 60(60%) were males and 40(40%) were females. Thus, the differences were statistically significant. More also, the age distribution of the respondents ranged from 16 years to 31 years and above, with a mean of 16.7 ± 3.5 years for smokers and 16.7 ± 8.6 years for non-smokers. In the population studied 34 (34%) and 30(30%) were within 16-20 years of age, 40(40%) and 52(52%) were within 21-25 and 26(26%) and 18(18%) were within 26-30 years of age for smokers and non-smokers respectively. While, there were more subjects in the smokers group within 26-30 and 16-20 years age group than in the non-smokers group. The prevalence of microorganisms isolated from the tooth brush of smokers in Ekpoma are Actinomyces odontolyticus (25.8%), Candida sp. (16.1%), Fusobacterium sp. (14.5%), Neisseria sp. (11.3%), Bacteroides sp. (9.7%), Lactobacillus acidophilus (9.7%), Enterobacter sp. (4.8%), Bifidobacterium sp. (3.2%), Eubacterium sp. (3.2%) and Klebsiella oxytoca (1.6%). While that isolated from the toothbrush of non-smokers in Ekpoma are Candida sp. (25%), Actinomyces odontolyticus (20.3%), Eubacterium sp. (17.2%), Neisseria sp. (15.6%), Bacteroides sp. (10.9%), Lactobacillus acidophilus (4.7%), Fusobacterium sp. (3.1%), Enterobacter sp. (1.6%) and E. coli (1.6%). Conclusively, the effects of these variables from this presents study found out that non-smokers has a higher prevalence than smokers.

Keywords: smokers and non-smokers, oral microbial floral and oral hygiene.

INTRODUCTION
Oral cavity does not maintain homogeneous conditions for colonization of microorganisms. The mucosal surface, such as lips, cheek and tongue as well as teeth has a distinct habitat of microbes due to their shapes, biological properties, conditions for growth of microbial communities. Human mouth or the oral cavity always remains bathed with saliva, which has a distinct effect on the ecology of mouth (Taylor and Francis, 2009). Microorganisms generally seen in the oral cavity include staphylococci, streptococci (Streptococcus mutans), Lactobacillus acidophilus, Actinomyces odontolyticus, anaerobic spirochetes and Vibrios (Marsh and Bradshaw, 1995).

Traditionally it has been cerebrated that mouth mucosa, the tongue, and the pharynx harbour characteristic bacterial pathogens causing chronic inflammation and focal infections.
The review emphasizes on the comparative study and the mechanisms of actions of the microorganisms present in the normal oral cavity and the patient suffering from oral cancers, and are detected in the preliminary stage. The entire discussion and description also co–relates the mode of action of the normal micro-flora of the normal oral cavity which when under certain circumstances changes to be opportunistic pathogens, and may trigger the genes which are having carcinogenic properties (Loesche, 1986). Smoking has been associated with an increase in morbidity and mortality due to atherosclerotic coronary artery disease, which has been confirmed in several studies (Kelly et al., 1975; Kannel, 1981; Robinson et al., 1988; Molstad, 1991; Gomez et al., 1993; Sahger et al., 1995; Gottlieb et al., 1996; Maggioni et al., 1998; Nicolau et al., 1998). This association results from the multiple noxious effects of smoking on the mechanisms of atherogenesis and thrombosis, and in the vasomotor and arrhythmogenic mechanisms (Sahger et al., 1995).

However, this study was aimed at assessing the occurrence and intensity of microbial floral present in the normal oral cavity of smokers and non-smokers in the study area.

MATERIALS AND METHOD

Area of Study

This study was carried out in Ekpoma. The administrative headquarter of Esan West Local Government Area of Edo State which lies between latitude 6.45°N to 6.75°N of the Equator and longitude 6.08°E to 6.13°E of the Greenwich Meridian with altitude of about 332m above sea level (Aziegbe, 2006). It is made up of quarters including Eguare, Iruekpen, Emaudo, Ujoelen, Ihumudumu, Illeh, Uke, Uhiele, Ujemen, Ukpenu, Idua, Ukhr, Egoro, Emehi, Igor and Idumebo (Aziegbe, 2006). Ekpoma has a population of 89,628 in 1991 and 127,718 in 2006, majority of which are civil servants, traders, business men/women, transporters, farmers, teachers/lecturers and students by occupation. A university (Ambrose Alli University) is situated in this town. The main sources of water in the locality are rainfall and wells. It is has 2 distinct seasons, wet and dry seasons. The wet season occurs between April and October with peck in August, average rainfall ranging 150cm to 250cm. The dry season occurs between November and March with cold harmatan between December and January, average temperature of about 25°C (Edo State of Nigeria, 1992).

Study Population

The study population of this research work comprises of both male and female individuals (smokers and non-smokers) with sample size (n=200) within age of 18 - 75 years in Ekpoma.

Sample Size

Samples (toothbrush) was collected from 200 individuals in Ekpoma of smokers (100) and non-smokers (100) randomly sampled.

\[
N = \frac{Z^2pq}{d^2},
\]

where

- \( N \) = the desired size
- \( Z \) = 1.96 (standard score)
- \( P \) = Prevalence (15%) (0.15) (Arowojolu et al., 2013).
- \( q \) = 1 - P (100-15=85%) (0.85)
- \( d \) = sample error tolerated (0.05)
\[ N = \frac{1.96^2 \times 0.15 \times 0.85}{0.05^2} = \approx 195.9 \text{ approximately } = 196 \]

**Inclusion and Exclusion Criteria**

Only subject samples of both male and female smokers and non-smokers who resides in Ekpoma were recruited for the study. While, subject samples of both male and female smokers and non-smokers who do not resides in Ekpoma, were not recruited for the study.

**Ethical Permission**

Ethical approval was obtained from the management (Ambrose Alli University, Ekpoma) and the informed consent of subject was also acquired before collection of samples.

**Collection of Samples**

Samples were collected from participants in the study by collecting their toothbrush preserved/kept in sterile container. The specimens on the toothbrush were inoculated and cultured in Blood agar, Sabouraud dextrose agar (SDA) and MacConkey agar. The culture plates were incubated at 37°C in a microbiological incubator for 24-72 hours. All procedures were carried out under standard aseptic conditions.

**Inoculation and Culture of Specimen**

The toothbrush of the participants was inoculated into peptone water, as well as Sabouraud Dextrose Agar (SDA) and incubated for 24hrs. Using sterile platinum wire loops (sterilized by flaming in a Bunsen flame), small portions of the overnight broth culture in peptone water of specimens from the toothbrush was picked and subculture. With a sterile wire loops, streaks were made on the culture media to achieve discrete colonies (Blood agar and MacConkey agar) (Cheesbrough, 2005). Appropriate biochemical tests were carried out to identify the bacterial isolates from the colony growth from the overnight culture.

**Statistical Analysis**

Percentage and chi-square test were used to analyze the data obtained from this study.

**RESULTS AND DISCUSSION**

A total of 200 participants, comprising of 100 (50%) non-smokers (control) and 100 (50%) smokers (cases), were used in the study. Previous studies have demonstrated that the oral microbes have several distinct microbial niches, e.g., the tooth surface, the subgingival sulcus, and oral mucosal surfaces (Winkelhoff *et al.*, 1985; Takahashi, 2005) which agrees with the present work. However, for the purpose of this study, and to simplify the problem, a non-smoker was classified as one who currently has not been smoking for at least one year prior to the commencement of the study. This was adopted for simplification reason as many of the respondents could not recall clearly when they stopped smoking and had limited education. There were, however, more males in the subject population than females, which was due to the fact that majority of the women did not consent to participate in the study. Majority of the women that declined to participate in the study opined that since the study directly relate to smoking habit, that it was not relevant to women. Amongst the (50%) subject smokers only two 4 (4%) were females and 96 (96%) were males while, out of the fifty (50%) of non-smokers 60 (60%) were males and 40 (40%) were females. Thus, the differences were statistically significant (p<0.05).

More also, result from table 4.1 shows the age distribution of the respondents ranged from 16 years to 31years and above, with a mean of 16.7 ± 3.5 years for smokers and 16.7 ± 8.6 years for non-smokers. In the population studied 34 (34%) and 30(30%) were within 16-20years of age,
40(40%) and 52(52%) were within 21-25 and 26(26%) and 18(18%) were within 26-30 years of age for smokers and non-smokers respectively. While, there were more subjects in the smokers group within 26-30 and 16-20 years age group than in the non-smokers group.

**Table 4.1:** Age and sex distribution of respondents according to their smoking status

<table>
<thead>
<tr>
<th>Smoking Status</th>
<th>Age Groups (%)</th>
<th>Sex (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16-20</td>
<td>21-25</td>
<td>26-30</td>
</tr>
<tr>
<td>Smokers</td>
<td>34 (34)</td>
<td>40 (40)</td>
<td>26 (26)</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>30 (30)</td>
<td>52 (52)</td>
<td>18 (18)</td>
</tr>
<tr>
<td>Total</td>
<td>64 (100)</td>
<td>92 (100)</td>
<td>44 (100)</td>
</tr>
</tbody>
</table>

The prevalence of microorganisms isolated from the tooth brush of smokers in Ekpoma as shown in Table 4.2 are *Actinomyces odontolyticus* (25.8%), *Candida spp.* (16.1%), *Fusobacterium spp.* (14.5%), *Neisseria spp.* (11.3%), *Bacteroides sp.* (9.7%), *Lactobacillus acidophilus* (9.7%), *Enterobacter sp.* (4.8%), *Bifidobacterium sp.* (3.2%), *Eubacterium sp.* (3.2%) and *Klebsiella oxytoca* (1.6%). While that isolated from the toothbrush of non-smokers in Ekpoma as shown in Table 4.3 are *Candida spp.* (25%), *Actinomyces odontolyticus* (20.3%), *Eubacterium sp.* (17.2%), *Neisseria sp.* (15.6%), *Bacteroides sp.* (10.9%), *Lactobacillus acidophilus* (4.7%), *Fusobacterium sp.* (3.1%), *Enterobacter sp.* (1.6%) and *E. coli* (1.6%).

**Table 4.2:** shows the prevalence of microorganisms isolated from the tooth brush of smokers in Ekpoma.

<table>
<thead>
<tr>
<th>Bacteria isolates</th>
<th>No. sampled</th>
<th>Prevalence rate (%)</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinomyces odontolyticus</em></td>
<td>32</td>
<td>25.8</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Enterobacter sp.</em></td>
<td>6</td>
<td>4.8</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Bacteroides sp.</em></td>
<td>12</td>
<td>9.7</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Neisseria sp.</em></td>
<td>14</td>
<td>11.3</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>2</td>
<td>1.6</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em></td>
<td>12</td>
<td>9.7</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Fusobacterium sp.</em></td>
<td>18</td>
<td>14.5</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Bifidobacterium sp.</em></td>
<td>4</td>
<td>3.2</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Eubacterium sp.</em></td>
<td>4</td>
<td>3.2</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Candida albican</em></td>
<td>20</td>
<td>16.1</td>
<td>1.00</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>100</td>
<td>0.476</td>
</tr>
</tbody>
</table>

Key: X² = chi-square *= pathogenic organisms

**Table 4.3:** shows the prevalence of microorganisms isolated from the tooth brush of non-smokers in Ekpoma.

<table>
<thead>
<tr>
<th>Bacteria isolates</th>
<th>No. sampled</th>
<th>Prevalence rate (%)</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinomyces odontolyticus</em></td>
<td>26</td>
<td>20.3</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Enterobacter sp.</em></td>
<td>2</td>
<td>1.6</td>
<td>1.00</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2</td>
<td>1.6</td>
<td>1.00</td>
</tr>
</tbody>
</table>
The result from this study is in line with previous work on the mean Oral Hygiene Indices (OHI) Smoking status of smokers and non-smokers, which shows that smokers generally had good oral hygiene than the non-smokers. However, there are some contrary studies that reported that smokers do not necessarily have better oral hygiene in comparison with their non-smoking counterparts (Brandtzæg and Jamison, 1964; Kristoffersen, 1970). Where it was reported that the mean OHI score was higher among smokers compared with the non-smokers. This finding can be explained by the fact that cigarette smoking causes staining of teeth, which roughens the surface of the teeth and encourages more rapid plaque accumulation. The findings of this study are similar to that of previous studies (Bastiaan and Waite, 1978; Feldman et al., 1983). Alexander, (1970) reported that accumulation of bacterial plaque was not associated with tobacco smoking among a group of students, a report that was corroborated by the report of Bastiaan and Waite, (1978) among young adults. The findings of this study could have been due to the fact that majority of the respondents were in the lowest socio-economic classes and had little education in comparison with the students studied by Alexander, (1970).

However, the comparison of smokers and non-smokers with similar level of oral hygiene have been studied with the finding that smoking parse has a marginal but significantly harmful effect on the periodontal tissue (Ismail et al., 1983). The gingivitis noted in the study by Skaleric and Kovac-Kavcic, (1999), was attributed to reduced gingival blood flow produced by nicotine in smokers.

The presence of cotinine and nicotine in saliva and crevicular fluid of smokers may have a profound effect on the tissue destruction seen in periodontal disease. The two indices used in this study (Oral Hygiene Index-System and Gingiva Index) as shown in table 4.4, were statistically significantly higher in the non-smokers. Since there were also statistically significant differences between smokers and non-smokers as regards sex and age, these variables could act as confounders on the effect of smoking on the indices.

**Table 4.4: Comparison of the mean values of oral hygiene indices according to their smoking status**

<table>
<thead>
<tr>
<th>SMOKING STATUS</th>
<th>Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oral Hygiene Index (OHI-S)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Smokers</td>
<td>8.67±5.61</td>
<td>0.05</td>
</tr>
<tr>
<td>Smokers</td>
<td>7.29±4.92</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Gingival Index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Smokers</td>
<td>4.00±5.20</td>
<td>0.05</td>
</tr>
<tr>
<td>Smokers</td>
<td>3.67±3.06</td>
<td>0.05</td>
</tr>
</tbody>
</table>
CONCLUSION
A major habitation of bacteria including micro-floras and opportunistic pathogens are present in the oral cavity. However, the resulting environment formed by the tobacco and nicotine consumptions, manipulates the homeostatic mechanism of the oral micro-flora, thus making a different niche of micro-organisms with pathogenic property. Therefore, the effects of these variables from this presents study found out that non-smokers has a higher prevalence than smokers i.e. Non-smokers is highly associated with Oral Hygiene Index-System and Gingiva Index compare to smokers. Therefore, this study concludes that smoking habits is deleterious to oral health. Detailed oral hygiene instructions should therefore be targeted at non-smokers and smokers should be encouraged to quit the habit.

RECOMMENDATIONS
It is therefore recommended that:
- Smoking habits is deleterious to oral health and smokers should be encouraged to quit the habit.
- Utmost oral hygiene instructions should be strictly adhere to by both subjects (smokers and non-smokers)
- The habits of all patients should be inquired about during an oral examination and the patients should be strongly advised to stop smoking by pointing out the risk.
- Treatment to periodontal diseases should be strictly adhering to rule out severe complications.
- Smokers should be encouraged to visit a dentist for preventive procedure more regularly than the non-smokers and better still, smokers should be encouraged to quit smoking as gingival disease is not without consequences if allowed to persist.

REFERENCES


