Comparative study of oral bacterial composition and neutrophil count between smokers and non-smokers

Ghadah Mohammed Saleh1*, Shaymaa Suhail Najim1, Azher Said Hindal1

ABSTRACT

Exposure to tobacco smoke has significantly impacted on the gingival and oral flora, therefore, this study was conducted to investigate the bacterial flora in the oral cavity of smokers and compare it with that of normal non-smokers. The study included 20 male smokers (mean age: 30 ± 7.2) and 20 male non-smokers (mean age: 31 ± 8.6). Bacterial isolates were identified after isolation by biochemical tests. The number of neutrophils in saliva was also estimated for all study individuals. The results revealed 171 bacterial isolate from smokers and non-smokers. The predominant isolate was Streptococcus sp. in both groups but with higher percentage in non-smokers (42.6%) compared to smokers (31.6%). Anaerobic bacteria showed a higher percentage (36.2%) in smokers compared to non-smokers (22.8%) at P < 0.05. Gram negative bacilli showed higher significant percentage in smokers (32.7%) compared to non-smokers (12.9%) (P < 0.05). The mean number of neutrophils in smokers was significantly lower (30 cell/ml) than non-smokers (48 cell/ml). Well-known pathogenic isolates presented only in oral samples of smokers. In conclusion, smoking may have a negative effect on neutrophils presence in the oral cavity and this may encourage the growth of opportunistic pathogenic bacteria to cause oral infections such as periodontitis as well as other infections.

Keywords: Neutrophil count, Normal flora, Oral cavity Bacteria, Smokers.

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INTRODUCTION

Oral health is a reflection of one general health affecting ability of a person to eat, speak, and contributes significantly to a sense of confidence and well-being [1]. Numerous studies have reviled a critical role for smoking in increasing the risk for developing extensive and severe forms of oral diseases; it increases the acquisition of periodontal pathogens and periodontal diseases, colonization by respiratory pathogens, and the occurrence of upper respiratory tract infection including otitis media [2]. Studies using molecular techniques for bacterial identification and characterization have showed that the oral microbial profile associated with periodontitis in smokers is diverse and distinct from that in non-smokers [3]. Bacteria colonize at tooth surface within a few minutes after its eruption into the oral cavity and begin to form complex communities [4]. Early colon-
ization of tooth-associated ecosystem is a specific and selective process, and the development of such community is effected by early inter bacterial as well as host-bacterium interactions [5]. Examining the effect of smoking on these nascent communities thus is an important initial step in understanding the itiopathogenic role of smoking in periodontal diseases. Human mouth has a constant bacterial presence that is kept under control, in part, by a continual influx of neutrophils from the surrounding periodontal tissues [6]. Neutrophils provide crucial defense functions against invading microorganisms and are a core element of innate immunity [7]. However, although their primary role is protective neutrophils release toxic products that are thought to be partly responsible for the destruction seen in periodontal diseases [8]. Therefore, the purpose of the present investigation was to compare bacterial acquisition and colonization in smokers and non-smokers with the detection of neutrophil count in the saliva of each individual.

MATERIALS AND METHODS

Study population
Thirty male smokers (mean age: 30 ± 7.2 years), 10 were neglected from the study depending on exclusion criteria and 20 male individuals who had never smoked, referred to here as non-smokers (mean age: 31 ± 8.6 years) were recruited in this study.

Exclusion criteria included
Smokers with systemic diseases that could affect immune function and neutrophil response such as, diabetes, presence of caries lesion or any kind of mucosal ulceration, use of immunosuppressant medications that may alter polymorphonuclear cells (PMN) number or steroids, antibiotic therapy and oral prophylactic procedures within the last 3 months.

Collection and counting of oral neutrophils
The protocol to collect and count oral neutrophils was a modification of that of Bender, et al. [9]. Briefly, patients were rinse their mouths with 10 ml of sterile hank saline. Hundred microliter of last dilution was spread onto blood agar base plates consisting 5% human blood, for isolation of aerobic and facultative bacteria, colombia blood agar with 5% blood, colistin, and nalidixic acid (to inhibit Gram negative bacteria) for isolating Gram positive anaerobes, tryptic soy agar with 5% blood, hemin, vitamin, and vancomycin (to inhibit most gram positive bacteria) for isolating Gram negative anaerobes, MacConkey agar plates for isolating enterobacteriacea members and sabouraud dextrose agar with chloramphenicol 0.005% and cyclohexamide 0.04% for isolating yeasts. All plates were incubated for 24 h at 37C° except the anaerobic culture plates were incubated anaerobically for 48 h at 37C°, further biochemical examination were performed for identification of bacterial isolates. Samples were also collected from subgingival sites by inserting a sterile endodontic paper point into each medial site for 10 second; the samples were then placed into eppendorf microfuge tubes containing 1 ml of sterile nutrient broth. Within half an hour the samples were mixed using a vortex for 30 second. Serial dilutions and inoculums was preformed the same as previously mentioned. VITEK system was used to confirm the identification of bacterial isolates.

Statistical Analysis:
Mean and standard deviation was used in the statistical analysis, Chi-square test was used to test significance comparison between percentages of groups. The statistical analysis was performed using the statistical analysis system-SAS 2012 program. Values were regarded as significantly difference at P < 0.05 level [10].

RESULTS AND DISCUSSION
From 50 subjects, 10 neglected from the study because the patients either used antibiotics or suffering diabetes. Thus, 20 smokers and 20 individuals who have never smoked were used in further experiments. The demographic characteristics of the subjects were shown in Table 1.

Table 1. Demographic characteristics and smoking status of participants. *, Mean ± standard deviation; **, P < 0.05.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-Smokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette consumption* (pack/day)</td>
<td>-</td>
<td>1.5 ± 0.71</td>
</tr>
<tr>
<td>Education :</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highly educated</td>
<td>18**</td>
<td>6</td>
</tr>
<tr>
<td>High school education</td>
<td>2</td>
<td>14 **</td>
</tr>
<tr>
<td>Mean age Years*</td>
<td>31 ± 8.6</td>
<td>30 ± 7.2</td>
</tr>
<tr>
<td>Duration of smoking (years)*</td>
<td>-</td>
<td>10 ± 2.4</td>
</tr>
</tbody>
</table>

It has been known that oral bacteria preferentially colonize on different surfaces in the oral cavity as a result of
specific adhesins on the bacterial surface binding to complimentary specific receptors on a given oral surface. The relation of such bacterial colonization with smoking has been a field for several studies [11-13]. Smoking may not ruin the teeth entirely, but weakened oral immune system caused by smoking, which increases the risk of infection. Smoking causes great changes to the mucous membranes in the mouth and growth conditions for the good bacteria deteriorate, resulting in an entirely different mouth flora, therefore the cultivable flora of non-smokers may show a complex of bacterial community [14]. As would be expected the highest bacterial load was consisted of normal oral flora, whereas an unexpected spectrum of pathogenic and opportunist microorganisms was found in the oral cavity of smokers including Gram-positive and negative bacteria as well as yeasts. A total of 171 isolates were identified from both smokers and non-smokers samples (Table 2).

Table 2. Microorganisms isolated from oral cavity of Smokers and Non-Smokers.

<table>
<thead>
<tr>
<th>Microbial isolates</th>
<th>Microbes in Smokers No (%)</th>
<th>Microbes in Non-smokers No (%)</th>
<th>Total Microbes No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus spp.</td>
<td>35 (31.8)</td>
<td>26 (42.6)</td>
<td>61 (35.6)</td>
</tr>
<tr>
<td>Viellonella spp.</td>
<td>3 (2.7)</td>
<td>12 (19.6)</td>
<td>15 (8.7)</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>15 (13.6)</td>
<td>6 (9.8)</td>
<td>21 (12.2)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>8 (7.2)</td>
<td>4 (6.5)</td>
<td>12 (7.0)</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>4 (3.6)</td>
<td>1 (1.6)</td>
<td>5 (2.9)</td>
</tr>
<tr>
<td>Actinomyces spp.</td>
<td>6 (5.4)</td>
<td>3 (4.9)</td>
<td>9 (5.2)</td>
</tr>
<tr>
<td>Heamophilus spp.</td>
<td>5 (4.5)</td>
<td>0 (0)</td>
<td>5 (2.9)</td>
</tr>
<tr>
<td>Neissera spp.</td>
<td>5 (4.5)</td>
<td>2 (3.2)</td>
<td>7 (4.1)</td>
</tr>
<tr>
<td>Fusobacterium spp.</td>
<td>5 (4.5)</td>
<td>0 (0)</td>
<td>5 (2.9)</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>6 (5.4)</td>
<td>2 (3.2)</td>
<td>8 (4.6)</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>3 (2.7)</td>
<td>0 (0)</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>7 (6.3)</td>
<td>3 (4.9)</td>
<td>10 (5.8)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>2 (1.8)</td>
<td>0 (0)</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>2 (1.8)</td>
<td>0 (0)</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>Unidentified</td>
<td>4 (3.6)</td>
<td>2 (3.2)</td>
<td>6 (3.5)</td>
</tr>
<tr>
<td>Total number of isolates</td>
<td>110 (64)</td>
<td>61 (35)</td>
<td>171</td>
</tr>
</tbody>
</table>

Streptococcus spp. was the predominant bacterial isolate in non-smokers compared to smokers (42.6 % vs 31.8%) (Fig 1). Anaerobic bacteria showed the highest percentage of isolation in all oral samples (63%) followed by aerobic bacteria 31% and the lowest was found in case of yeasts (6%) (P < 0.05) (Fig 2). Fig 3 shows that the distribution of aerobic and anaerobic bacteria showed that smokers had a higher percentage of aerobic bacteria 36.2% compared to non-smokers 22.8%, whereas, anaerobic bacteria had a higher percentage in non-smokers 71.9% as compared with smokers 57.3% (P < 0.05).

The yeast isolation showed no significant difference in both smokers and non-smokers. Smoking may increase the temperature of the oral cavity to 42°C, causing the denaturation of proteins in the mucous membrane.
It may also decrease the oxygen tension in periodontal pockets which initiate a good environment for anaerobic pathogenic bacterial growth [15]. The most predominant organism was *Streptococcus* spp and *Staphylococcus* spp., which had a higher percentage (47.8%) of isolation than other Gram negative cocci including *Neisseria* spp. and *Veillonella* (12.8%) in all oral samples recruited in the study, also Gram negative bacilli showed higher percentage (25%) than Gram positive bacilli (8%) (P < 0.05, Fig 4).

![Fig 4. Percentage of Gram positive and negative (cocci and bacilli) bacteria in oral samples of all individuals of the study.](image)

Gram negative bacilli showed higher significant percentage in smokers (32.7%) compared to non-smokers (12.9%) (P < 0.05), whereas Gram negative cocci was significantly higher in percentage (19.8%) in non-smokers compared to smokers (2.7%) (P < 0.05) as shown in Fig 5.

![Fig 5. Percentages of Gram positive and negative (cocci and bacilli) bacteria in oral samples of both smokers and non-smokers subjects.](image)

The mean number of neutrophils in smokers was 30 cell/ml whereas, in non-smokers was significantly higher 48 cell/ml at (P< 0.05; Fig 6). The decreased number of neutrophils in smokers group can be explained by the toxicity effect of smoking cigarettes on neutrophil cells because this smoke contain toxic chemical compounds that may cause the death of neutrophils , and this also explain the higher numbers of bacterial colonization especially the pathogenic species which is unaffected with cigarette smoke (Fig 7).

![Fig 6. Neutrophil count (cell/ml) in both smokers and non-smokers subjects.](image)

Studies indicated tobacco smoke contain 28 toxic compound as nicotine, carbon monoxide, carcinogenic benzoic and others in elevated levels which alter both innate and acquired immune response [16], as well as increasing the risk of cancer and many heart and pulmonary diseases [17,18]. Some reports indicated the increase in susceptibility to infection by smoking as a result of changes in the humoral and cellular immune response [19].

![Fig 7. Percentage of bacterial isolates (pathogenic) presented only in smokers.](image)

Molecular byproducts of smoking interfere with mechanisms that normally control growth of damaging bacteria at the surface of the oral mucosa in gingival crevices. In this way smoking can promote early development of oral infections [20]. The flora of smokers contains fewer aerobic and anaerobic organisms with interfering activity against bacterial pathogens and harbors more potential pathogens as compared with the flora of non-smokers [21]. Therefore, change in microflora induced by smoking would provide a certain advantage to colonization by a subset of pathogens [22,23]. The main source of neutrophils in the oral cavity is from those migrating from the gingival sulcus [22]. Nicotine in cigarette smoke has been reported to cause an increase in saliva excretion rates followed by a
decrease rate, it also cause a decreased buffer effect and increased saliva pH [24]. Smoking affect both local and general immune responses, by changing chemotaxis and phagocytosis of polymorphonuclear cells, altering antibody response, inhibiting T-lymphocyte proliferation, etc. [25]. In conclusion smoking is not only the main cause of heart and lung diseases, but also a strong factor to encourage the maintenance and growth of pathogenic bacteria and as a result a cause of periodontitis and other oral diseases.

Conflict of interest

The authors declare that they have no conflict of interests.

REFERENCE


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