Periodontal Health Status and Microbiological Analysis of Orthodontic Patient in Dhaka Bangladesh

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ABSTRACT

Aim: The aim of the study was to determine the level of bacteria in dental plaque associated with fixed orthodontic appliances.

Materials and Methods: This longitudinal observational study was done during April’2014 to September’2014 in the Department of Microbiology, Primeasia University, Banani, Dhaka. The 100 samples were collected purposively from the Department of Orthodontics, University Dental College and Hospital, Dhaka and reported private Orthodontic clinic of Bangladesh. The microbiological test was done in the Department of Microbiology, Primeasia University, Banani, Dhaka.

Results: The study result showed, there were significantly increased plaque accumulation as well as microorganisms in patient who wear fixed orthodontic appliances than normal subjects. Most of the organisms were streptococcus and most organisms were sensitive to antibiotic except eromycin, sensitive to paste (triclosan and fluoride) but resistant to mouth wash.

Conclusion: The components of fixed appliance can hamper proper oral hygiene maintenance increases the chance of accumulation of plaque that lead to caries. So appropriate tooth brush with proper paste can inhibit microbial colonization on tooth surfaces.

Key Words: Orthodontic appliance ; Microbiological analysis; Periodontal health

INTRODUCTION

Orthodontics is concerned with correcting or improving the position of teeth and correcting any malocclusion. Day by day, the popularity of Orthodontic treatment is increasing in the world as well as in Bangladesh. Most of the orthodontic cases are noninvasive and health risk is low.1 However, these appliances can be associated to difficulty in cleaning and serve as different impact zones and modify microbial adherence and colonization, acting as foreign reserves and possible sources of infection.2,3 Good oral hygiene maintenance, such as adequate tooth brushing, mouth rinsing and dental flossing, plays a vital role in maintaining healthy teeth, especially in the orthodontic patients.4,5 The placement of fixed orthodontic appliances generally impede good oral hygiene, and the appliance component can cause alteration in oral micro flora by reducing pH, increasing affinity of bacteria to the metallic surface because of electrostatic reactions, and causing retention areas for microorganism. Thus, they lead to plaque accumulation around the bracket base. Plaque retention surrounding orthodontic appliances leads to enamel demineralization caused by organic acids produced by bacteria in the dental plaque.5-9

Plaque is tenaciously adherent deposit that forms on tooth surfaces. It consists of an organic matrix containing dense concentration of bacteria. In microbiological terms, plaque is a biofilm. Biofilms consist of a hydrated viscous phase formed from bacteria and their extracellular polysaccharide matrices. Plaques become visible, particularly on the labial surfaces of the incisors, when tooth brushing stopped for 12 – 24 hours.10

Figure 1: Dental Plaque

Fixed orthodontic appliances create new retention areas, which are suitable for bacterial colonization and lead to an the absolute number and percentage of Streptococcus mutans and lactobacilli.4,11,12 Forsberg et al,11 evaluated microbial colonization of 12 patients treated by fixed orthodontic
appliances and reported that the lateral incisor attached to the archwire with an elastomeric ring exhibited a greater number of microorganisms in the plaque than teeth ligated with steel wire. They also reported a significant increase in the number of S. mutans and lactobacilli in the saliva after the insertion of fixed appliances. They recommended that the use of elastomeric ligation rings should be avoided in patients with inadequate oral hygiene because elastomeric ligation rings will significantly increase microbial accumulation on tooth surfaces adjacent to the brackets, leading to a predisposition for the development of dental caries and gingivitis.

More than 600 bacterial species comprise the plaque microflora that exists on surfaces within the oral cavity. Caries-related bacteria, including Streptococcus mutans and Lactobacillus, and periodontitis-associated bacteria, such as Eubacterium, Fusobacterium, and Treponema, have been detected in orthodontic appliance-associated dental plaque.

Triclosan, a chlorophenol derivative, kill germs by interfering with the enzymes required for fatty acid synthesis. Similar to triclosan the fluorinated products were also found to possess marked antibacterial activities. These active compounds were reducing cariogenic bacteria to strengthen the teeth by reducing demineralization and increasing remineralization of teeth.

To date, there is no comprehensive studies have been carried out in Bangladesh to compare the bacterial level of dental plaque in subjects with or without fixed orthodontic appliance. Therefore the aim of the study was to determine the level of bacteria in dental plaque associated with fixed orthodontic appliances.

MATERIALS AND METHODS

100 patients were selected purposively who are selected for fixed orthodontic treatment. Subjects age should have 11 to 25 years, no history of previous orthodontic treatment, Presence of all teeth except 3rd molars. Patient should co-operative and should have healthy periodontium. Subjects who have severe crowding were excluded from the study.

According to selection criteria 100 subjects were selected purposively. At the beginning and 3 months after orthodontic treatment of each subject, plaque was collected with sterile probe from 1st molars and or premolars and transferred to sterile test tube containing 5 ml of normal saline. Samples were stored in cold place and transported to the Laboratory.

Score Criteria for Plaque

0  No plaque
1  A film of plaque adhering to the free gingival margin and adjacent area of the tooth, which cannot be seen with the naked eye. But only by using probe
2  Moderate accumulation of deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen with the naked eye
3  Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

One hundred micro liter of undiluted samples were collected with micro pipette and spread on the surface of Plate count-agar media using sterile spreader. Cultures were incubated anaerobically for 24 hrs at 37°C. Count of more than 20 colonies were considered as positive samples.

After estimation of positive samples on the surface of plate count-agar medium, small colonies were subcultured on the surface of blood-agar plates for further purification and incubated anaerobically for 24 hours at 37°C. The following methods were used for initial characterization of the isolates:

1. Colonial shape and form on plate count-agar and blood agar.
2. Gram-staining and microscopic examination
3. Biochemical test
   a. Voges- Proskauer (VP) test b. Methyl Red (MR) test
4. Antibiotic sensitivity

At first prepare a Muller Hilton Broth, then inoculated colonies and incubate for 6 hours at 37°C. Then collected the sample from broth with sterile swab stick and spread to Muller Hilton agar plate. In the plate hole was prepared on the centre of the plate for control point and on the periphery for different antibiotics. In control point hole filled with sterile water and peripheral hole filled with different antibiotics (10 times diluted) and incubated for 24 hours at 37°C.

Antibiotic used in this study: Amoxicillin, Cephadrine, Cefuroxime, Cefixime, Doxycycline, Clindamycin, Metronidazol, Flucoxacilline, Ciprofloxacain, Arithromycin, Amoxicilnine + clavulanic acid

5. Mouth wash and paste sensitivity

Mouth wash was diluted with same amount of water (1:1) and 1 gm paste was mixed with 1 ml of water. a Muller Hilton Broth was prepared then inoculated colonies and incubated for 6 hours at 37°C. Then collected the sample from broth with sterile swab stick and spread to Muller Hilton agar plate. In the plate hole was prepared on the centre of the plate for control point and on the periphery for different paste and mouth wash. In control point hole filled with sterile water and peripheral hole filled with different antibiotics (10 times diluted) and incubated for 24 hours at 37°C.

Mouth wash used in this study: Arodine, Oroclean Paste used in this study: Pepsdent Gum care, Pepsdent Sensitive, Pepsdent Germ check, Meryl Baby gel, Darlie baby gel, Paradontex, Sensodyne, Close up
Periodontal Health Status and Microbiological Analysis of Orthodontic Patient in Dhaka Bangladesh

RESULTS

**Table: 1 Microbiological analysis**

<table>
<thead>
<tr>
<th>Pt Name</th>
<th>Gram staining</th>
<th>Methyle Red test (MR)</th>
<th>VP test</th>
<th>MSA Media</th>
<th>Blood agar Media</th>
<th>Tentative Microorganism</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Shape</td>
<td>Arrangement</td>
<td>Gram reaction</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Afsana 1</td>
<td>Cocci</td>
<td>Single</td>
<td>Gram+ve</td>
<td>ND</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>Afsana 2</td>
<td>Cocci</td>
<td>Cluster</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Afsana 3</td>
<td>Large rod</td>
<td>Chain</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ β-hemolysis</td>
</tr>
<tr>
<td>Riva 1</td>
<td>Cocci</td>
<td>Chain</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Riva 2</td>
<td>Cocci</td>
<td>Chain</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Riva 3</td>
<td>Rod</td>
<td>Single</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Rozy 1</td>
<td>Rod</td>
<td>Single</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Rozy 2</td>
<td>Cocci</td>
<td>Cluster</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Rumi 1</td>
<td>Cocci</td>
<td>Single</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Rumi 2</td>
<td>Diplococci</td>
<td>Diploid</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Mou 1</td>
<td>Cocci</td>
<td>Single</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Mou 2</td>
<td>Cocci</td>
<td>Cluster</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Shumi 1</td>
<td>Rod</td>
<td>Single/Chain</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
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<tr>
<td>Shumi 2</td>
<td>Rod</td>
<td>single</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
<tr>
<td>Nabila</td>
<td>Rod</td>
<td>chain</td>
<td>Gram+ve</td>
<td>ND</td>
<td>ND</td>
<td>+ α-hemolysis</td>
</tr>
</tbody>
</table>

Table: 1 Shows Most of the bacteria are **Streptococcus** and some are staphylococcus.

Figure: 3.  Alfa Hemolysis on Blood Agar Media

Figure 4. Beta Hemolysis

Figure 3 Shows Greenish Alfa hemolytic Zone

Figure 4 Shows Clear Beta hemolytic zone
Figure-5: Number of the patient: Pie chart shows 68% subjects are female and 32% are male.

Figure-6: Age wise distribution of Subjects
Total 100 (18.38+ 4.06 years) Male 32 (18.97+ 4.42 years) Female 68 (18.17 + 3.89)

Figure shows all age group carry more or less equal subjects, Female subjects are more in all age group but most in 16-20 years age group.

Table: 2. Brushing habit of male and female according to age group.

Table: 3. Use of mouthwash by the subjects according to age group

Table: 4. Shows plaque accumulation significantly increases during orthodontic treatment. Plaque accumulation more in male than female in both group.

Table: 5. Effects of Tooth Brush on Plaque accumulation

Table: 6. Effects of Mouth wash on Plaque accumulation

Table: 7. Bacterial Count - CFU/mg (Before Orthodontic Treatment – CFU 1 and During Orthodontic treatment – CFU2)
Table: 8. Effects of tooth brush on bacterial colonization

<table>
<thead>
<tr>
<th></th>
<th>CFU/µg (mean)</th>
<th>CFU/µg (mean)</th>
<th>Difference CFU/µg</th>
<th>Std. Deviation</th>
<th>t-value</th>
<th>P-value</th>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Once daily</td>
<td>1.70 X10⁶</td>
<td>1.26 X10⁵</td>
<td>0.44 X10⁵</td>
<td>0.83 X10⁴</td>
<td>2.517</td>
<td>0.020</td>
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<tr>
<td>Twice daily</td>
<td>1.08 X10⁶</td>
<td>0.94 X10⁵</td>
<td>0.14 X10⁵</td>
<td>0.32 X10⁴</td>
<td>1.387</td>
<td>0.21</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Once daily</td>
<td>1.15 X10⁷</td>
<td>0.99 X10⁶</td>
<td>0.25 X10⁶</td>
<td>0.57 X10⁵</td>
<td>3.443</td>
<td>0.01</td>
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<tr>
<td>Twice daily</td>
<td>0.68 X10⁶</td>
<td>0.50 X10⁵</td>
<td>0.18 X10⁵</td>
<td>0.32 X10⁴</td>
<td>1.387</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once daily</td>
<td>1.29 X10⁷</td>
<td>0.99 X10⁶</td>
<td>0.30 X10⁶</td>
<td>0.65 X10⁵</td>
<td>4.256</td>
<td>0.000</td>
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<tr>
<td>Twice daily</td>
<td>0.94 X10⁶</td>
<td>0.78 X10⁵</td>
<td>0.16 X10⁵</td>
<td>0.79 X10⁵</td>
<td>3.866</td>
<td>0.001</td>
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Table: 9. Effects Mouthwash on Microbial colonization

<table>
<thead>
<tr>
<th>Mouthwash</th>
<th>CFU/µg (mean)</th>
<th>CFU/µg (mean)</th>
<th>Difference CFU/µg</th>
<th>Std. Deviation</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.90 X10⁷</td>
<td>0.72 X10⁶</td>
<td>0.18 X10⁶</td>
<td>0.21 X10⁵</td>
<td>2.914</td>
<td>0.080</td>
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<tr>
<td>No</td>
<td>1.26 X10⁷</td>
<td>1.65 X10⁶</td>
<td>0.39 X10⁶</td>
<td>0.97 X10⁵</td>
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<td>0.051</td>
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<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.67 X10⁷</td>
<td>0.53 X10⁶</td>
<td>0.14 X10⁶</td>
<td>0.28 X10⁵</td>
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<tr>
<td>No</td>
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<td>0.90 X10⁶</td>
<td>0.25 X10⁶</td>
<td>0.57 X10⁵</td>
<td>3.498</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>0.78 X10⁷</td>
<td>0.62 X10⁶</td>
<td>0.16 X10⁶</td>
<td>0.24 X10⁵</td>
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<td>1.00 X10⁶</td>
<td>0.30 X10⁶</td>
<td>0.71 X10⁵</td>
<td>3.886</td>
<td>0.000</td>
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Table : 9. Shows Bacterial count decreases who use mouthwash.

Figure: 15. shows Most of the organism are sensitive to antibiotic. Some bacteria are more sensitive to ciprofluoxetine, cephradin, and clindamycin

Figure: Antibiotic sensitivity test

Figure: shows Bacteria are sensitive to antibiotic
showed Plaque Index significantly increased after insertion of fixed orthodontic appliance. Before Orthodontic therapy, Plaque Index (PLI A) was 1.18 and after insertion of fixed appliance Plaque Index (PLI B) increased to 1.73. Similar study conducted by U. Hagg et al.\textsuperscript{17} and found a 10 percent mean increase in the plaque index after insertion of Fixed orthodontic appliance. Similar changes in plaque accumulation during orthodontic treatment with removable appliances\textsuperscript{18,19} and Fixed orthodontic appliances have been reported by several authors.\textsuperscript{20,21}

Scheie et al. also found the similar result and concluded that, the presence of rough surfaced bonding materials acting as a plaque trap may have played a contributory factor.\textsuperscript{22}

In contrast to these observation, Sinclair et al.\textsuperscript{23} showed no significant difference in plaque accumulation between pre-treatment and the insertion of Fixed orthodontic appliance. This finding has been supported by Davies et al.\textsuperscript{24} who evaluated the occlusal status, dental health and socio-psychological development. They concluded that, behavioral factors rather than the orthodontic treatment itself were responsible for the additional gain in oral hygiene and gingival health experienced by the patients fitted with orthodontic appliance. However, a survey performed by Kwan\textsuperscript{25} in Hong Kong from lower income groups, demonstrated a significant increase in Plaque index after insertion of a Fixed orthodontic appliance, confirming the earlier hypothesis that in addition to the patient’s attitude and behavior, social class may also be a contributory factor in controlling plaque accumulation. Hence the significant increase in the Plaque Index after insertion of fixed orthodontic appliance in present study could partly be due to the patient’s attitude and behavior, as well as the presence of Fixed orthodontic appliance which made it difficult to keep the teeth clean.

Possibly as a consequence of the increased plaque index, a concomitant increased bacterial count of 0.38 X 10^5 CFU/mg was noted after insertion of fixed orthodontic appliance in the present study. Similar results were found by U. Hagg, Rosenbloom et al. and Sukuntapatipark et al.\textsuperscript{26,27}

This study shows most of the antibiotic and paste were sensitive against bacteria and plaque accumulation decreased who brush their teeth twice daily and who use mouth wash. The treatment with fixed appliance may alter the ecological environment in the oral cavity by introducing new stagnant areas available for bacterial colonization. The appliance may interfere with proper oral hygiene practice. Proper teeth brushing and use of mouth wash can control plaque accumulation as well as bacterial colonization.

**CONCLUSION**

Based on the observation of the study, in conclusion followings are suggested:

1. The components of fixed appliance increases the chance of accumulation of plaque as well as Bacterial colonization.
2. Plaque accumulation less who brush their teeth twice daily.
3. Appropriate tooth brush with proper paste can inhibit microbial colonization on tooth surfaces.

**References**

3. Hayder F. Salomo, Harraa S. Mohammed-Salih and Shaymaa F. Rasheed, The influence of different types of fixed orthodontic appliance on the growth and adherence of microorganisms (in vitro study)


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