Assessment of Salivary Secretory Immunoglobulin A (sIg A) Level during Fixed Orthodontic Treatment

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ABSTRACT

Background: The immune system of the oral cavity suffers alterations due to fixed orthodontic treatment which act as potent stimulus for oral secretory immunity. The aims of this study are to estimate the effect of fixed orthodontic appliance on the level of salivary sIgA at different time intervals, and to verify the gender difference.

Materials and method: The patient's history, clinical examination, and fixed orthodontic appliances were placed for 30 Iraqi orthodontic adult patients had class II division 1 and/or class I malocclusion (15 males and 15 females) aged 18-25 years old. The undiluted whole saliva was collected from each sample immediately before wearing fixed appliance (control group T0 as base line), and after 2 weeks (T1), 1 month (T2), and 1 year (T3) of wearing fixed orthodontic appliance. The levels of salivary sIgA were measured by Enzyme Linked Immunosorbant Assay kit (ELISA).

Results: The mean value of salivary sIgA was elevated at T1 and reached the peak at T2 followed by declined at T3 to reach near the normal value at T0 (base line). Repeated measure ANOVA test showed statistically highly significant difference among four time intervals. The Bonferroni test after repeated measure ANOVA test showed highly statistical significant difference between each two time intervals except between T0 and T3 show significant difference. In addition there were no significant gender differences.

Conclusion: In this study one can conclude that fixed orthodontic appliance acts as an immunological stimulant in the oral cavity that changes the level of salivary sIgA which evaluate the immunity status in the oral cavity.

Key words: Saliva, Salivary sIgA, fixed orthodontic treatment. (J Bagh Coll Dentistry 2016; 28(3):149-154).

INTRODUCTION

Saliva is important body fluid and exceptional compound which composed of a number of systems which work for a wide spectrum of physiological needs to guard the oral mucosa and the entire body from infection (1,2).

Immunoglobulin A (IgA) is an antibody that has a critical role in mucosal immunity. The production of IgA in mucosal lining is more than all other kinds of antibody together, 3-5 gm IgA are secreted into the intestinal lumen per day (3).

Mucosal secretory Immunoglobulin A (sIgA) is formed through two distinctive pathways, namely T cell dependent and independent pathway (4). The sIgA can withstand the cruel environment of gastrointestinal tract and be responsible for defense against microbes that reproduce in body secretions because the secretory component of sIgA keeps the immunoglobulin from being fragmented by proteolytic enzymes (5).

Also sIgA can inhibit inflammatory effects of other immunoglobulins and it is a poor activator of the complement system and opsonizes just weakly (6). The sIgA antibodies avoid adherence and penetration of antigens and high level of sIgA could avoid allergen absorption, while low levels of sIgA and transient IgA deficiency have been associated with an improved possibility for allergy and bronchial hyper-reactivity (7).

Secretory IgA is a secretory factor for acquired immunity in the oral cavity. Antibodies of this type play a part in the maintenance of the integrity of the oral surfaces (enamel and mucous membrane) and become part of first line of defense through limiting the microbial adhesion.

The sIgA antibodies independently, or in complexes, take part in antigen-antibody reactions on the mucous membrane (and partly on the enamel too), thus restrict the penetration of bacteria and toxins (8-10). The largest amount (90%) of sIgA is synthesized by the parotid and submandibular salivary glands. The plasma cell of these glands secrete dimeric immunoglobulin A, that relates with a secretory particle to proteolysis, and secreted by the epithelial cells of the acini (11).

Recognition of sIgA antibodies in saliva may aid to identify liable patients before development of orthodontically induced root resorption (12). The tipping movements is the easiest type of orthodontic tooth movement (OTM) which are formed when single force is employed toward the crown of the tooth, for the duration of this movement the PDL are compressed (bone resorption) adjacent to the root apex on the similar side of the applied force and the crest of alveolar bone on the opposed side. In bodily movement, bone resorption takes place along side the whole alveolar surface on the pressure side, while bone deposition takes place along side the alveolar surface on the tension side (13).

The state of calcium metabolism in alveolar bone affects the tooth movement that is directly applicable to orthodontics. In addition the bone metabolism occurs in the alveolar process and basilar bone of law (14).

The calcium hemostasis is a procedure which preserves the mineral equilibrium, which is...
associated by their temporarily related mechanisms 1. quick flow of calcium from bone (within few seconds) 2. little term response of osteoclast and osteoblast (from minutes to days)  

At starting of orthodontic tooth movement, the mechanical stimulus lead to an acute inflammatory response inside the periodontal tissues, subsequently initiates the biologic processes which result in bone resorption to provide accommodations of the tooth movement  

In addition, orthodontic treatment is very often related to gingival inflammation, as a consequence of the local alteration in microbial ecosystem and in the content of the bacterial plaque. During orthodontic treatment, patients with good oral hygiene may develop gingival inflammation. When fixed orthodontic appliances are used, mild to moderate gingivitis with gingival enlargement and bleeding on probing is obvious.

The inflammatory cell infiltration on periodontal tissues occurs due to forces of orthodontic movement, formed signals and cytokines for differentiation and activation of clast cells. The chronic inflammatory process can promote the production of autoantigens to the immune system and break the immunological tolerance. The salivary glands secreted large amounts of secretory IgA (sIgA) into saliva, which is the main line of defense of the oral cavity and upper respiratory tract surfaces.

The aims of the current study are estimation of water, then freeze until analysis. Each patient rinsed his mouth several times with water and waited 1-2 minutes for clearance of water, then sat in a restful position on the chair and instructed to open his lips slightly. The patients asked to drool passively unstimulated saliva over the lower lip into sterile plane plastic test tube. The patients instructed not to spit into test tube and not to swallow during saliva collection.

**Exclusion criteria**

The exclusion criteria are none of the patients reported acute or chronic inflammatory or autoimmune diseases, systemic disease, previous facial and orthodontic surgical treatment, previous trauma of the primary or permanent teeth, smoking, or the usage of steroidal and non-steroidal anti-inflammatory medications for at least a month before sampling, pregnancy and lactating patients, clinical signs of periodontal disease, periapical lesions, or root resorption, and oral mucosa lesions or active caries before bonding.

**Orthodontic materials (Figure 1-A)**

Stainless steel orthodontic brackets Roth 0.022 (OrthoTechnology), molar tubes (OrthoTechnology), ligature wire (preformed ligure Ties shorty 0.10 inch Ortho Technology), light cure orthodontic adhesive (OrthoTechnology), nickel titanium arch wire 0.014 inch round (Ortho Technology) for T1 and T2, and 0.021x0.025 inch rectangular stainless steel wire (OrthoTechnology) for T3.

**Saliva collection and storage**

The saliva sample in this study was collected at fixed time between 9 A.M.-12 P.M. The collection period was 10 minutes and the patients were instructed not to drink or eat or chew gum (except water) at least 1 hour before saliva collection.

Each patient rinsed his mouth several times with water and waited 1-2 minutes for clearance of water, then sat in a restful position on the chair and instructed to open his lips slightly. The patients asked to drool passively unstimulated saliva over the lower lip into sterile plane plastic test tube. The patients instructed not to spit into test tube and not to swallow during saliva collection.

The plane test tube(Figure 1-B) with 5 ml unstimulated saliva samples were collected, coded, organized in the rack, and stored in cooling box containing ice containers after collection to stop growth of bacteria, then the samples were carried out to immunologic laboratory in the teaching laboratories of Baghdad medical city, at the same day the coded test tube with saliva sample centrifuged at 3000 rpm for 10 minutes(Figure 1-C) and then immediately the clear supernatants layer was collected by adjustable pipette(Figure1-D) into labeled eppendorf tubes which were set in eppendorffs rack (Figure 1-E )and stored at -20º C in a deep freeze until analysis.
Saliva was collected from each patient at baseline T0 (immediately before fixed orthodontic appliance wearing), T1 (after 2 weeks of fixed orthodontic appliance wearing), T2 (after 1 month of fixed orthodontic appliance wearing), and T3 (after 1 year of fixed orthodontic appliance wearing).

The level of salivary secretory slgA was measured by Enzyme Linked Immunosorant Assay Kit (ELISA) (27) Demeditec Secretory IgA ELISA kit (DEXK276) Germany (Figure 1-F), Microplate ELISA washer (Figure 1-G), BIO-RAD Microplate ELISA reader Australia (Figure 1-H) and printer of ELISA reader (Figure 1-I). The ELISA test procedures were reagent preparation and assay procedure according to ELISA kit instructions. The optical density measured at 450 nm, the photometer blank was set on the first calibrator then point by point method was used for data reduction using calculating factor (1.0) to calculate analyte factor for saliva as mentioned in instructions for use of slgA ELISA kit.

Calculation of results (Table 1)
As mentioned in slgA ELISA kit instructions, the slgA concentration (µg/ml) in saliva samples was obtained by:
1. Calculate the mean absorbance values (OD450) for each pair of calibrators and samples
2. Plot a calibration curve OD on the Y-axis versus secretory IgA concentration on the X-axis.
3. Determine the corresponding concentration of slgA (µg/ml) in unknown samples from the calibration curve, and then computerized data reduction was applicable. Point- by- point or linear data reduction is recommended.

| Table 1: Calculation of the result (Demeditec slgA ELISA kit instructions) |
|------------------|---------------------|
| Calibrators      | Concentration Value (µg/ml) | Absorbance units (450mn) |
| CAL1             | 0                    | 0.10                  |
| CAL2             | 2                    | 0.14                  |
| CAL3             | 20                   | 0.33                  |
| CAL4             | 40                   | 0.57                  |
| CAL5             | 100                  | 1.03                  |
| CAL6             | 400                  | 2.17                  |

Statistical analysis
Data were collected and statistically analyzed by a software computer program SPSS (statistical package of social science) software version 15 for windows XP Chicago, USA. The following statistics were used:
A. Descriptive Statistics: means, standard deviations, minimum and maximum values.
B. Inferential Statistics: including the following test:
1. Independent t-test: to compare statistically the mean value of slgA level for each time interval between both genders.
2. Repeated measure ANOVA test: to test any statistically significant difference among time intervals for the mean value of slgA level.
3. Bonferroni test: to compare between each two time intervals of slgA level when ANOVA test showed a statistically significant difference.

In the statistical evaluation, the following levels of significance are used:
- Non-significant NS P > 0.05
- Significant S 0.05 ≥ P > 0.01
- Highly significant HS P ≤ 0.01

RESULTS AND DISCUSSION
Salivary slgA can be considered as a marker for evaluation of immune status in the oral cavity during fixed orthodontic treatment. The descriptive statistics of each gender and gender difference using independent t-test for the mean values of salivary slgA level (µg/ml) at different time intervals T0, T1, T2, and T3 showed in table (2). The descriptive statistics of total sample for the mean value of salivary slgA level (µg/ml) at different time intervals showed in table (3). The comparison of the salivary slgA level (µg/ml) among T0, T1, T2, and T3 was done using repeated measure ANOVA test as shown in table (4), followed by Bonferroni test for the measurements that showed significant difference as shown in table (5).

As revealed in table (2), the independent t-test showed non-significant gender difference, this agreed with Eliasson et al. (28) and Youness et al. (29), so sample was pooled in table (3).

Table (3) and Figure (2) showed descriptive statistics of total sample for the mean value of salivary slgA level (µg/ml) at different time intervals. The mean value of slgA level elevated following fixed orthodontic appliance in total sample at T1 and reached the peak at T2 followed by a decline at T3 to reach near the normal value at T0, these elevations of the mean value of slgA level at T1 and T2 might be caused by the innate immune response after fixed orthodontic appliance wearing which had some cytokines that affect the slgA production and delivery on the mucosal surface. The orthodontic force induced Interleukin-1 (IL-1) and Tumor necrosis factor-alpha (TNF-α) which are inflammatory cytokines.
of the innate immune response \((22,30-32)\), both cytokines can motivate sIgA delivery from mucosal surface and exchange throughout the epithelial barriers and induce clast differentiation and activation \((33,34)\). Cytokines, such as IL-4, IL-5, IL-10, and TGF-ß favouring antibody production and inhibiting clast activation \((24,33,35)\). Salivary sIgA could control local clast activation. However, a local inflammatory response could induce an imbalance in the autoimmune response and could tend to activation of clast cell \((12)\).

During orthodontic tooth movement compression areas and hyaline necrosis in the periodontium may damage the cementum layer and expose the dentin matrix \((23)\), the damaged periodontal tissue causes inflammation which can result in reorganization of antigen-presenting cells and can lead to the expression of co-stimulatory molecules that tend more to lymphocytes activation \((36,37)\).

The orthodontic appliance materials were exposed to microbial adhesion that decrease oral hygiene and produce new retentive areas for plaque and debris and possibility of subsequent infection. Fixed orthodontic appliance stimulates continuous accumulation and retention of microbial growth. Recent reports suggest that is difficult to eliminate the microbial growth or maintain the hygienic status of the fixed orthodontic appliances at the critical zones \((38)\). The metal of orthodontic appliance inside the oral cavity can cause increase in concentration of metal ions which cause increase in biofilm biomass \((39)\). The orthodontic biomaterial components release potential allergens such as metal ions from the base metal alloys in orthodontic fixed appliances, and resin based bonding materials, the intra-oral orthodontic materials may lead to pathomorphological variations in the mouth and antigen stimulation

Fixed orthodontic appliances composed from many metallic ions as chromium, cobalt, and nickel. These metallic ions and monomers released from orthodontic adhesive materials have a strong effect on oral secretory immunity \((41)\), nickel is a strong immunologic sensitizer, and nickel sensitivity is lower in subjects with orthodontic treatment \((42)\).

Table (4) was repeated measure ANOVA test for comparing the salivary sIgA level \((\mu g/ml)\) among T0, T1, T2, and T3. The results showed highly significant difference among time intervals.

Table (5) was Bonferroni test which showed highly significant difference between each two time intervals (T0 and T1, T0 and T2, T1and T2, T1 and T3, T2 and T3) except between T0 and T3 where it was significant difference. The salivary sIgA level declined at T3 to reach near the normal value at T0, so there is significant difference between T0 and T3, this result may be due to that patients with long duration fixed orthodontic treatment perhaps they develop immunological tolerance \((42)\).

So in this study one can conclude that fixed orthodontic appliance acts as immunological stimulant in the oral cavity which change the level of salivary sIgA at different time intervals that evaluate the immunity status of the oral cavity, because fixed orthodontic appliances after two weeks and four weeks of wearing cause highly significant difference in the level of salivary sIgA in both genders during active orthodontic treatment, while after 1 year the level of salivary sIgA return nearly to the normal value.

The current study is a unique study for determination the sIgA level \((\mu g/ml)\) at these time intervals T0, T1, T2, and T3 during fixed orthodontic treatment.
Table 2: Descriptive statistics of each gender and gender difference for salivary sIgA (µg/ml) level

<table>
<thead>
<tr>
<th>Duration</th>
<th>Gender</th>
<th>Descriptive statistics</th>
<th>Genders' difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>T0</td>
<td>Males</td>
<td>15</td>
<td>137.84</td>
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<tr>
<td></td>
<td>Females</td>
<td>15</td>
<td>138.80</td>
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<tr>
<td>T1</td>
<td>Males</td>
<td>15</td>
<td>373.39</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>15</td>
<td>373.82</td>
</tr>
<tr>
<td>T2</td>
<td>Males</td>
<td>15</td>
<td>478.41</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>15</td>
<td>478.50</td>
</tr>
<tr>
<td>T3</td>
<td>Males</td>
<td>15</td>
<td>140.15</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>15</td>
<td>141.43</td>
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</table>

Table 3: Descriptive statistics of total sample for salivary sIgA (µg/ml) level

<table>
<thead>
<tr>
<th>Duration</th>
<th>N</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>S.D.</th>
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<tbody>
<tr>
<td>T0</td>
<td>30</td>
<td>133.04</td>
<td>144.87</td>
<td>138.32</td>
<td>3.84</td>
</tr>
<tr>
<td>T1</td>
<td>30</td>
<td>367.91</td>
<td>379.75</td>
<td>373.61</td>
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<tr>
<td>T2</td>
<td>30</td>
<td>472.07</td>
<td>484.69</td>
<td>478.46</td>
<td>4.08</td>
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<tr>
<td>T3</td>
<td>30</td>
<td>135.14</td>
<td>146.45</td>
<td>140.79</td>
<td>3.43</td>
</tr>
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</table>

Table 4: Repeated measure ANOVA test for sIgA level at different time intervals

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>F-test</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td>Sphericity Assumed</td>
<td>2627026.757</td>
<td>3</td>
<td>875675.586</td>
<td>80897.115</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Greenhouse-Geisser</td>
<td>2627026.757</td>
<td>2.833</td>
<td>927408.305</td>
<td>80897.115</td>
<td>0.000**</td>
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<tr>
<td></td>
<td>Huynh-Feldt</td>
<td>2627026.757</td>
<td>3</td>
<td>875675.586</td>
<td>80897.115</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Lower-bound</td>
<td>2627026.757</td>
<td>1</td>
<td>2627026.757</td>
<td>80897.115</td>
<td>0.000**</td>
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<tr>
<td>Error (factor1)</td>
<td>Sphericity Assumed</td>
<td>941.737</td>
<td>87</td>
<td>10.825</td>
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<tr>
<td></td>
<td>Greenhouse-Geisser</td>
<td>941.737</td>
<td>82.147</td>
<td>11.464</td>
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<tr>
<td></td>
<td>Huynh-Feldt</td>
<td>941.737</td>
<td>87</td>
<td>10.825</td>
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<tr>
<td></td>
<td>Lower-bound</td>
<td>941.737</td>
<td>29</td>
<td>32.474</td>
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Table 5: Bonferroni test

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<th>p-value</th>
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<tr>
<td>T0</td>
<td>-235.28</td>
<td>0.000**</td>
</tr>
<tr>
<td>T1</td>
<td>-340.14</td>
<td>0.000**</td>
</tr>
<tr>
<td>T2</td>
<td>-2.47</td>
<td>0.026*</td>
</tr>
<tr>
<td>T3</td>
<td>-104.85</td>
<td>0.000**</td>
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REFERENCES