Effect of orthodontic tooth movement on salivary levels of Interleukin-1beta, Tumor Necrosis Factor-alpha, and C-reactive protein

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ABSTRACT

Background: Orthodontic force is considered to stimulate cells in the periodontium to release many mediators such as cytokines which play a responsible role for periodontal and alveolar bone remodeling, bone resorption and new bone deposition. Aim of this study was carried out to estimate changes of the (interleukin-one beta, tumor necrosis factor - alpha and C-reactive protein) levels in unstimulated whole saliva during the leveling stage of orthodontic tooth movement.

Materials and methods: The sample consisted of thirty adult patients (12 males and 18 females) with ages ranges (19-23) years. Each sample had Class I and Class II malocclusion dental classification and required bilateral extraction of their maxillary first premolars, underwent a session of professional oral hygiene and received oral hygiene instructions before and during the period of study, one month later fixed orthodontic appliance were placed in the upper arch by using 0.014 nickel titanium arch wire. The unstimulated whole saliva was taken from each sample immediately before placement of the appliance (baseline), and at (after1hour, after one week and after two week) following placement of the fixed orthodontic appliance. In addition the plaque index and gingival index were recorded during the interval periods of this study to assess oral cleanliness. The interleukin – one beta and tumor necrosis factor-alpha were determined by enzyme linked immunosorbent assay, while the C-reactive protein was determined by latex agglutination.

Results: The results of the present study found the mean value of both salivary (interleukin-one beta and tumor necrosis factor -alpha) were highest at (after1hour) followed by at (after one week) then at (after two week) than the baseline with highly statistical significant differences (P< 0.01) among males, females and total samples, in addition there were no statistical significant differences between males and females (P>0.05). While the mean values of C-reactive protein were higher at (after 1hour) only with highly significant differences (P< 0.01) among females and total samples while only significant difference (P<0.05) for males, in addition there were no statistical significant differences between males and females. Regarding the correlation between salivary (interleukin – one beta and tumor necrosis factor -alpha), there were positive correlation between them at all periods of study. Moreover there were positive correlation between salivary (interleukin – one beta and tumor necrosis factor-alpha) and salivary C-reactive protein. On the other hand there were no association between the salivary (interleukin – one beta and tumor necrosis factor-alpha and C-reactive protein) and clinical parameter (plaque index and gingival index).

Conclusion: From this clinical study we conclude that orthodontic force induces increasing the levels of (interleukin – one beta, tumor necrosis factor -alpha, C-reactive protein) in unstimulated whole saliva during orthodontic tooth movement.

Keywords: interleukin-one beta, tumor necrosis factor - alpha, C-reactive protein, unstimulated saliva, orthodontic tooth movement. (J Bagh Coll Dentistry 2013; 25(4):120-125).

INTRODUCTION

Orthodontic tooth movement is based on force that stimulate periodontal ligaments and alveolar bone remodeling, at the early stage of orthodontic tooth movement, the host response to orthodontic force has been described as an aseptically and transitory inflammation characterized by alteration the vascularity and blood flow of periodontal ligament (PDL), resulting in local synthesis and release of different mediators such as cytokines (IL-1β, TNF-α, etc.) involved in alveolar bone remodeling process (1–4). Importantly, IL-1β is pro-inflammatory cytokines produced by the periodontal ligament (PDL) in sufficient quantities to diffuse into the gingival crevicular fluid (GCF) and has been identified as a biomarker of orthodontic tooth movement (5–8).

TNF-α is a typical mediator of inflammatory response that has been shown to be involved in the process of bone resorption and to be locally elevated in response to orthodontic force. It plays a prominent role in the mechanism controlling the appearance of osteoclasts at compression sites (8–14). Furthermore cytokines such as (IL-1β) and TNF-α produced at the gingival sites may be transported into the systemic circulation and stimulate hepatocytes in the liver to produce C – reactive protein (CRP), which provide a biomarker for low grad systemic inflammation (15–19). Inflammatory cytokines such as IL-1β and TNF-α which are involved in bone and periodontal remodeling, have been quantified in the salivary crevicular fluid of patients undergoing orthodontic treatment (7–8,11,20). Different mediators involved in alveolar bone remodeling are continuously washed into saliva by GCF. whole-saliva samples may constitute an easy alternative to individual gingival sulcular
samples for determining analytes of bone turnover that are present within the periodontal environment, providing a sensitive and inexpensive detection technique (21).

Aim of this study was carried out to estimate changes of the (interleukin-one beta, tumor necrosis factor – alpha and C-reactive protein) levels in unstimulated whole saliva during the leveling stage of orthodontic tooth movement.

MATERIAL AND METHODS
Subjects recruited for the study were volunteer patients attending the orthodontic department at the College of Dentistry University of Baghdad seeking for orthodontic treatment. A total of 30 adult patients (12 males and 18 females) with an age range of (19-23 years) were participated in the study. They should be with Class I and /or Class II malocclusion cases requiring bilateral extraction of their maxillary first premolar teeth. Each subject prior the placement of the orthodontic appliance should extracted both upper first premolars (right and left) at least 20 days. During this period, all the subjects should be with good oral hygiene (22). The collection of the unstimulated salivary samples from the individuals was formed under standardized conditions (23-24). In the current study saliva collected between 9-12 am at different times; before placement of the orthodontic appliance(1hour) as a baseline (T0), then 1 hour after placement of orthodontic appliance (T1), one week (T2), two week (T3). The samples were stored at -20ºc in a deep freeze until processed (25).

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IL-1β has been pre-coated onto a microplate. Standards and samples were pipetted in to the wells and any IL-1β present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-1β was added to the wells. After washing, Avidin conjugated Horseradish peroxidase (HRP) was added to the wells. Following a washed to remove any unbounded Avidin-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of IL-1β bound in the initial step. The color development was stopped and the intensity of the color was measured. C-reactive protein in saliva was detected by using latex agglutination slide test (Human Tex CRP) for the qualitative and semi-quantitative determination of C-reactive protein in non-diluted saliva.

**Principle:** Human tex CRP was based on the immunological reaction between human C-reactive protein (CRP) of patient specimen or control serum and the corresponding anti-human CRP antibodies bound to latex particles. The positive reaction is indicated by distinctly visible agglutination of the latex particles in the test cell of the slide. All data analyses were performed using the SPSS statistical software program (version 10 for Windows, SPSS). The confidence level was accepted at the level of 5%.

RESULTS
Results showed that the mean value of salivary IL-1β (pg/ml) and TNF-α (pg/ml) were highest at T1, followed by T2, then T3 and then T0 with highly statistically significant difference (P< 0.01) among males, females, and total sample as shown in (table 1) and (table 2) respectively. According the T-test, the mean value of salivary IL-1β (pg/ml) among males was higher than females with no statistically significant difference (P > 0.05) at T0, T1, T2, and T3. The same results was recorded concerning TNF-α (pg/ml).

**Table 1. Mean of IL-1β concentration (pg/ml) among samples**

<table>
<thead>
<tr>
<th></th>
<th>Baseline (T0)</th>
<th>After 1 hour (T1)</th>
<th>After 1 week (T2)</th>
<th>After 2 weeks (T3)</th>
<th>Mean</th>
<th>SD</th>
<th>T-test</th>
<th>Mean</th>
<th>SD</th>
<th>T-test</th>
<th>Mean</th>
<th>SD</th>
<th>T-test</th>
<th>ANOVA Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>No.</td>
<td>Mean</td>
<td>SD</td>
<td>T-test</td>
<td>Mean</td>
<td>SD</td>
<td>T-test</td>
<td>Mean</td>
<td>SD</td>
<td>T-test</td>
<td>Mean</td>
<td>SD</td>
<td>T-test</td>
<td>F</td>
</tr>
<tr>
<td>Males</td>
<td>12</td>
<td>161.99</td>
<td>6.19</td>
<td>0.005</td>
<td>163.02</td>
<td>22.29</td>
<td>0.43</td>
<td>260.64</td>
<td>31.02</td>
<td>0.005</td>
<td>214.35</td>
<td>19.02</td>
<td>0.38</td>
<td>233.52</td>
</tr>
<tr>
<td>Females</td>
<td>18</td>
<td>186.82</td>
<td>7.31</td>
<td>0.005</td>
<td>174.07</td>
<td>27.78</td>
<td>0.38</td>
<td>284.37</td>
<td>18.99</td>
<td>0.005</td>
<td>216.33</td>
<td>16.04</td>
<td>0.005</td>
<td>216.52</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>158.89</td>
<td>7.25</td>
<td>0.005</td>
<td>167.79</td>
<td>22.41</td>
<td>0.38</td>
<td>255.08</td>
<td>12.98</td>
<td>0.005</td>
<td>219.54</td>
<td>11.24</td>
<td>0.005</td>
<td>217.59</td>
</tr>
</tbody>
</table>

No significant= P value > 0.05. *(Significant)= P value < 0.05.**(Highly significant)= P value < 0.01.
As demonstrated in Table 3 the mean value of salivary CRP (mg/l) was highly at the T1 with highly statistically significant difference (P<0.01) among females and total sample and only significant difference (P<0.05) for males. The present study reported the normal of salivary CRP at the period (T0, T2, and T3) for both males and females. According to T-test, the mean value of salivary TNF-α (pg/ml) among females was higher than males with no statistically significant difference (P > 0.05) at T1.
DISCUSSION
Orthodontic tooth movement is a biological process of bone remodeling, involving an acute inflammatory response in the periodontal tissues, characterized by increasing in vascular permeability and cellular infiltration of leukocytes [2,13]. Some cytokines such as IL-1β, TNF-α play important role in regulation of bone remodeling during orthodontic tooth movement, through recruitment of osteoclast precursors from the circulation, maturation and activation, in addition they promote osteoclast formation and activation [26-28]. The result of the present study showed that the mean value of salivary level IL-1β and TNF-α were elevated to reach the peak at T1 following placement of the orthodontic appliance during the study periods followed by declined at T2 then T3. This elevation at the 1 hour might be caused by an early upregulation of chemotactic activities directly after continuous mechanical force application and this in agreement with previous findings [5,7,8,29,30]. While disagreement with other studies [6,31] who were demonstrating no change in the IL-1β and TNF-α at the (1 hour) in the GCF in orthodontic tooth movement, this may be due to the variations in the study design, and these differences exist in terms of sample size, patients’ gender, age and salivary sample type, when many of these studies did not included these factors in the multivariate analysis. Concerning to the declining the level of the cytokines at the T2 and T3 of this study might be to the activity of the anti-inflammatory cytokines such as IL-10 which is known immune regulatory functions, including suppression of the proinflammatory cytokines and stimulation that play a role in bone resorption and periodontal tissue destruction [32]. The present study found no significant differences between males and females, this in agreement with Serra et al. [33] who found no differences in enzymztic activity during orthodontic tooth movement between males and females. The result of the present study revealed synergistically correlation between the IL-1β and TNF-α at all the period times of this study (T0, T1, T2, and T3), this in agreement with Dinarello [34].

Several studies found that salivary CRP may largely reflect local inflammation in the mouth [35-36], adapted to these studies the salivary level of CRP among samples were measured in the present study. The results of this study illustrated the highest level of the CRP at the (T1) with non-significant differences between males and females. Concerning to the correlation between (IL-1β and CRP) and (TNF-α and CRP), there were significant correlation in positive direction at the (T1), this can be rationalize by the transport of the cytokines (IL-1β and TNF-α) from the gingival sites into the systemic circulation which stimulate the hepatocytes in the liver to produce CRP and increase their level, this in agreement with the previous studies [15,17]. The normal of the CRP at the T0 indicated that the patients involved in this study were healthy. In additional, normal of the CRP at the T2 and T3 can be rationalized by decreasing and cease the stimulation effect of the bone resorption, this in agreement with Haheim [37] who demonstrated that the production of CRP related to the stimulation, that the level of CRP falls rapidly when the stimulation is ceased. The result of the present study shown no significant differences between males and females; this

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Table 5. Correlation coefficient (r) between means of IL-1β and CRP among samples

<table>
<thead>
<tr>
<th>Gender</th>
<th>No.</th>
<th>IL-1β and CRP</th>
<th>Baseline (T0)</th>
<th>After 1 hour (T1)</th>
<th>After 1 week (T2)</th>
<th>After 2 week (T3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>12</td>
<td></td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
</tr>
<tr>
<td>Females</td>
<td>18</td>
<td></td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td></td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
</tr>
</tbody>
</table>

*(significant correlation) = P value < 0.05. **(highly significant correlation) = P value < 0.01.

Table 6. Correlation coefficient (r) between means of TNF-α and CRP among samples

<table>
<thead>
<tr>
<th>Gender</th>
<th>No.</th>
<th>TNF-α and CRP</th>
<th>Baseline (T0)</th>
<th>After 1 hour (T1)</th>
<th>After 1 week (T2)</th>
<th>After 2 week (T3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>12</td>
<td></td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
</tr>
<tr>
<td>Females</td>
<td>18</td>
<td></td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td></td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
<td>r P-Value</td>
</tr>
</tbody>
</table>

*(significant correlation) = P value < 0.05. **(highly significant correlation) = P value < 0.01.
finding come in agreement with the finding of Serra et al. (33) who found that between males and females there was no differences in enzymatic activity during orthodontic tooth movement.

REFERENCES

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