Evaluation of Salivary Interleukin-1beta (IL-1β) Level in Relation to the Periodontal Status in Smoker and Non-smoker Individuals
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Abstract
Background: Smoking is the major risk factor in the periodontal diseases. Interleukin-1 (IL-1), have been associated with the immunopathology of periodontitis. IL-1β has been particularly studied as a critical determinant of tissue destruction in periodontitis.

Patients and Methods: The sample comprised 80 male volunteers (40 smokers and 40 non-smokers); aged 25-40 year old. Personal information was obtained from all subjects. Periodontal condition was measured including plaque index, bleeding index and clinical attachment level and bone loss. The assay of IL-1β was performed by enzyme linked immunosorbent assay. Chi square test, t-test and analysis of variance (ANOVA) were used to analyze the data.

Results: The reported mean value of plaque index was significantly higher among non smoker, and smoker periodontitis (1.72, and 1.87, respectively) than non-smoker, and smoker healthy groups (0.44, and 0.64 respectively) (P<0.000). The mean value of bleeding on probing was significantly higher among non-smoker and smoker periodontitis (0.22, and 0.06 respectively) than non-smoker and smoker healthy groups (0.04, and 0.01 respectively) (P<0.000). The mean value of IL-1β was significantly higher among smoker and non-smoker periodontitis (525.8 pg/ml, 357 pg/ml, respectively) than group of smoker and non-smoker healthy persons (124 pg/ml, 81.5 pg/ml, respectively) (P<0.000). Smokers with periodontitis reported significantly higher CAL than non-smokers with periodontitis (3.02 and 2.5, respectively) (p value ≤ 0.05). The bone loss was significantly higher among smokers with periodontitis than non-smoker with periodontitis (2.6, 2.14, respectively) (P value=0.04). The correlation between salivary IL-1β and PI, BOP, CAL, bone loss was significant (r=0.773, 0.335, 0.941, 0.939 respectively) (correlation <0.001) (for all clinical parameters).

Conclusions: Positive association was observed between periodontal diseases and smoking, salivary IL-1β were positively associated with clinical signs of periodontal disease and it appears to serve as biomarker of periodontitis.

Keywords: Interleukin 1beta; Periodontal disease; Periodontitis; Smoking

Introduction
Periodontal diseases are infectious diseases in which periodontopathogens trigger chronic inflammatory and immune responses that are thought to determine the clinical outcome of the disease [1]. Smoking is the major risk factor in the prevalence, extent and severity of periodontal diseases [2]. Cross-sectional studies have shown that smokers are two to seven times more likely to present periodontitis, compared to non-smokers [2,3]. Clinical studies have demonstrated that smokers have more severe periodontal disease, with increased bone loss [4] greater periodontal attachment loss, more gingival recession and periodontal pocket formation [5]. The presence of periodontopathogens, such as Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola (called the red complex) and Aggregatibacter actinomycetemcomitans, considered the major etiologic agents in periodontitis [6], triggers the expression of proinflammatory cytokines, such as interleukin-1 (IL-1), which have been associated with the immunopathology of periodontitis [7]. IL-1β has been particularly studied as a critical determinant of tissue destruction due to its proinflammatory and bone resorptive properties, the levels of the proinflammatory cytokine IL-1β are characteristically increased in diseased periodontal tissues and are thought to be a critical determinant of periodontitis outcome [8].

The hypothesis to be tested was that whether there is any relationship between salivary IL1 beta levels and clinical findings in smokers and non-smokers and to assess usefulness of IL-1 beta for diagnosis of periodontal severity.

Patients and Methods
This study comprised 80 male volunteers (40 smokers and 40 non-smokers); aged between 25-40 year old who were visiting the department of oral medicine and periodontics at school of dentistry, university of sulaimani along 9 successive months (September 2009-May 2010) for seeking dental treatment. This research was approved by Committee of Ethics at Research of the University of Sulaimani. According to declaration of Helsinki, signed consent forms were obtained from all participants before conducting the study [9]. Personal information was obtained from all subjects including age, past and present medical history, drug history, oral hygiene measurements, and habit of smoking. Subjects were classified into 3 categories according to their reported smoking habit, namely, “smoker” (smokes every day), “former smoker”, and “never been smoker”. “Former smoker” and “never been smoker” were defined as “non-smokers” in data analyses.

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as light smokers and heavy smokers based on this equation Pack years=(No. of cigarette smokers per day×No. of years smoked)/20. Light smokers: a pack year score ≤ 5, Heavy smokers: a pack year score ≥ 5. The smoker groups divided into four groups as follows: Healthy Light Smoker [11] (HLS), Healthy Heavy Smoker [10] (HHS), Periodontitis Light Smoker [11] (PLS) and Periodontitis Heavy Smoker [12] (PHS). Subjects excluded from the study were those suffering from any acute or chronic medical conditions including diabetes or any systemic diseases, viral, fungal or bacterial infections. Subjects suffering from aggressive periodontitis, periodontal abscess, periapical abscess, and those who had received periodontal treatment or antibiotic within preceding 3 months.

Subjects must not have less than 20 teeth in their mouth and they were equally divided into four groups as follows: Non-smokers and free from periodontal disease (NH), Smokers and free from periodontal disease (SH), Non-smokers with chronic generalized periodontitis (NP) and Smokers with chronic generalized periodontitis (SP). Healthy subjects include those with maintenance of a functional periodontal attachment level; minimal or no recession in the absence of interproximal bone loss [13]. Periodontal diagnosis was based on the classification of American Academy of Periodontology AAP [14]. Patients with at least 30% of sites with loss of attachment were classified as having generalized chronic periodontitis and radiographic evidence of bone loss.

Three indices were applied to measure the periodontal condition for all present teeth except the 3rd molar including plaque index (PI), bleeding index (BI) and clinical attachment level (CAL). The score of each index was derived by examining 4 sites of each tooth (mesial, buccal, distal and lingual).

The dental plaque was assessed according to plaque index. It ranged from 0-3 [15] to describe the amount of plaque on tooth surface.

Assessment of bleeding was done immediately after the PI measurement by using Williams’ periodontal probe and passing it to the base of the pocket. Four surfaces of each tooth were assessed [16], on probing. The percentage of BOP was measured by summation of surfaces with the bleeding divided to the number of surfaces present multiplied by 100.

Clinical attachment level was assessed by measuring the distance from cemento-enamel junction (CEJ) to the alveolar crest height, to the nearest millimetre was recorded using calliper was used for bone loss measurement. The distance from the CEJ to the alveolar crest height, to the nearest millimetre was recorded for each interdental area. If the distance exceeded 2mm in oblique and/or horizontal direction it was considered as resorption [18]. If bone loss could not be measured the site was regarded as unreadable. The bone loss of each patient was obtained by the summation of bone loss of anterior and posterior segments, sites that had bone loss where divided to the number of sites that have bone loss. Mesial and distal site of upper and lower first permanent molars and central incisors had been taken for measurement of bone loss.

Unstimulated whole expectorated saliva was collected from each subject according to the method described by Navazesh [12] and Zhang et al. [19] the subjects were refrained from eating and draining for at least 1/2 hour prior to the sampling, subjects was asked to rinse their mouth with tap water, tilt their head foreword and then expectorate whole saliva in to a sterile container without swallowing for 5 min at 9-11 AM before doing periodontal examination. Collected samples were placed on ice packs immediately, then transported to the laboratory and centrifuged at 5000 rpm for 10 minutes. The supernatant structure was kept frozen at -80°C as aliquots until assayed. Freezing and thawing cycles were avoided.

The assay of IL-1β was performed by ELISA using human (II1 beta) kit and quantitative sandwich immunoassay technique. The sample analysis was done in biochemistry laboratory, Maternity Teaching Hospital, Sulaimani.

The sample size was calculated using the following formula
\[ n = \frac{N(1+N)(e/2)^2}{(N=100, e=0.05}. \]
Statistical analysis was performed using Spss programme version 13, using chi square test, T-test and analysis of variance (ANOVA); the rejection level for the null hypothesis was less than 0.05 (5%).

Results
The study comprised 80 male volunteers (40 smokers and 40 non-smokers); aged between 25-40, they were categorized into three age groups 25-29 years, 30-34 years, 35-40 years, in which nearly 70% of studied sample were between 30–40 year old.

There were no statistically significant differences between all groups regarding all clinical parameters (II1B, plaque index, bleeding on probing, clinical attachment level and bone loss (Table 1).

Regarding PI, the reported mean value was significantly higher among non-smoker and smoker periodontitis (1.72 and 1.87, respectively) than non-smoker, and smoker healthy groups (0.44 and 0.64, respectively) (P=0.000). Regarding bleeding index (BI) the mean value was significantly higher among non-smoker and smoker periodontitis (0.22 and 0.06, respectively) than non-smoker and smoker healthy groups (0.04 and 0.01, respectively). (P=0.000). The mean value of IL-1β was significantly higher among smoker and non-smoker periodontitis (525.8 pg/ml and 357 pg/ml, respectively) than group of smoker and non-smoker healthy persons (124 pg/ml and 81.5 pg/ml, respectively) (P=0.000) (Table 2).

In smoker and non-smoker subjects with periodontitis, the mean value of PI was not statistically significant (1.87, 1.72) (P value ≥ 0.05), among subjects with periodontitis, the mean value of bleeding on probing was significantly higher among non-smokers than smokers (0.22, 0.06 respectively) (P=0.000). The clinical attachment levels
were calculated for both smokers and non-smokers with periodontitis where smokers with periodontitis reported significantly higher CAL than non-smokers with periodontitis (3.02 and 2.5 respectively) (p value ≤ 0.05). The bone loss was significantly higher among smokers with periodontitis than non-smokers with periodontitis (2.6 and 2.14, respectively) (P value=0.04). Among periodontitis subjects, the mean value of IL1 β was significantly higher among smokers than non-smokers (525 and 357 pg/ml, respectively) (p=0.000) (Table 3).

Discussion

In this study PI of non-smoker and smoker periodontitis were not significant (1.93, 1.81 respectively) (P value ≥ 0.05). The value of bleeding on probing was higher in light smokers than heavy smokers with periodontitis (0.711 and 0.553, respectively); however, the association was not statistically significant (p value ≥ 0.05). The mean value of clinical attachment level was higher among heavy smoker than light smoker (3.02 and 2.7, respectively); however, the association was not statistically significant (p value ≥ 0.05). The mean value of bone loss was higher among heavy smokers than light smoker (2.8 and 2.4 respectively), however, the association was not statistically significant (P value ≥ 0.05). Heavy smoker with periodontitis were reported higher mean value of IL1 β than light smoker with periodontitis (334.1, 314.20, respectively), however, the association was not statistically significant (p value ≥ 0.05) (Table 4). In healthy persons, nearly comparable mean value of IL1 β was reported among light and heavy smokers (126 and 122 respectively), the association between variables were not significant (p=0.65) (Table 5). The PI, BOP, CAL, Bone loss were significantly correlated with elevated level of IL-1β. The high value of these parameters was positively related with elevated level of IL-1β, the correlation between salivary IL-1β and PI, BOP, CAL, bone loss were significant (r=0.773, 0.335, 0.941, 0.939 respectively) (correlation <0.001) (for all clinical parameters) (Table 6).

BOP is of value for the early diagnosis and prevention of periodontal disease and used as a predictor of periodontal breakdown in retrospective and prospective clinical studies [11]. In this study, mean value of bleeding index was higher among non-smoker and smoker periodontitis than non-smoker and smoker healthy groups. This association was statistically highly significant. Calsina et al. [5] in their study on effects of smoking on periodontal tissues they found that among cases, bleeding on probing was less evident in smokers than in non-smokers. Mahuca et al. [21] evaluated the degree of periodontal disease and its relationship to smoking habits in a population of young healthy male Spanish military recruits. They report higher bleeding indices in non-smokers, similarly, in this study, non-smoker persons with periodontitis had reported higher BOP than smoker persons with periodontitis, moreover, the heavy smoker groups showed lower value of bleeding index than light smoker groups this finding could be explained due to nicotine induced vasoconstriction in smoker’s gingiva as well as heavy gingival keratinization in smokers [22].

Effect of smoking on alveolar bone and CAL has been studied by several authors. Luzzi et al. [23] previously reported a relationship between alveolar bone loss and tobacco consumption. The findings when they investigated the relationship between cigarette smoking and bone loss in a group of dental hygienists were suggestive of an effect on alveolar bone that was independent of plaque levels. They also reported that this relationship was age-related, suggesting that progression was more significant in younger smokers. Calsina et al. [5] in their study on effects of smoking on periodontal tissues they found that among cases, clinical attachment level was greater in smokers than in former smokers or non-smokers, there was a dose-effect relationship between cigarette consumption and the probability of having advanced periodontal disease. Mahuca et al. [21] evaluated the degree of periodontal disease and its relationship to smoking habits in a population of young healthy male Spanish military recruits. They reported greater attachment loss were in smokers. Some studies have also highlighted the dose relationship between the effect of cigarette consumption and periodontal attachment loss [24]. The possible explanation is the cumulative effect of smoking on periodontal microbial, immune impairment and cytokine up-regulation. Similarly, in this study smoker individuals with periodontitis were reported high CAL and bone loss value than non-smoker persons with periodontitis. Moreover, the CAL and bone loss were higher among heavy smoker persons than light smokers.

In this study, the mean value of IL-1β was highly significant in subjects with periodontitis than in healthy ones. When compared with other studies the figure was the same, Orozco et al. [25] showed that IL-1β can act on a large number of cells (fibroblasts, chondrocytes, bone cells, neutrophils and lymphocytes) as well as IL-1beta concentrations and Non smoker Individuals. J Interdiscipl Med Dent Sci 2: 120. doi: 10.4172/2376-032X.1000120

Page 3 of 5

<p>| Table 1: Explores different ages of studied groups in relation to periodontal measures. |</p>
<table>
<thead>
<tr>
<th>Age group</th>
<th>IL1 β (pg/ml)</th>
<th>P value</th>
<th>PI M ± SD</th>
<th>P value</th>
<th>BOP M ± SD</th>
<th>P value</th>
<th>CAL (mm) M ± SD</th>
<th>P value</th>
<th>Bone loss M ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-29</td>
<td>258.3 ± 162.9</td>
<td>0.390</td>
<td>1.1 ± 0.7</td>
<td>0.08</td>
<td>0.10</td>
<td>1.2 ± 1.3</td>
<td>0.475</td>
<td>1 ± 1.1</td>
<td>0.418</td>
<td></td>
</tr>
<tr>
<td>30-34</td>
<td>243.2 ± 194.6</td>
<td>0.390</td>
<td>1.2 ± 0.7</td>
<td>0.08</td>
<td>0.10</td>
<td>1.2 ± 1.4</td>
<td>1.6 ± 1.6</td>
<td>1.4 ± 1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-40</td>
<td>309.1 ± 205.1</td>
<td>0.390</td>
<td>1.1 ± 0.8</td>
<td>0.09</td>
<td>0.13</td>
<td>1.2 ± 1.4</td>
<td>1.6 ± 1.6</td>
<td>1.4 ± 1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<p>| Table 2: Mean ± SD among studied group. (†)Indicates statistically significant result p&lt;0.05. |</p>
<table>
<thead>
<tr>
<th>Code</th>
<th>IL1 β (pg/ml)</th>
<th>PI M ± SD</th>
<th>BOP M ± SD</th>
<th>CAL (mm) M ± SD</th>
<th>Bone loss M ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smoker Healthy</td>
<td>81.5 ± 51</td>
<td>0.44 ± 0.4</td>
<td>0.04 ± 0.10</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Smoker healthy</td>
<td>124 ± 16.9</td>
<td>0.64 ± 0.4</td>
<td>0.01 ± 0.0</td>
<td>2.5 ± 0.78</td>
<td>2.14 ± 0.76</td>
</tr>
<tr>
<td>Non-smoker periodontis</td>
<td>357 ± 61.7</td>
<td>1.72 ± 0.3</td>
<td>0.22 ± 0.1</td>
<td>2.5 ± 0.78</td>
<td>2.14 ± 0.76</td>
</tr>
<tr>
<td>Smoker periodontis</td>
<td>525.8 ± 67.1</td>
<td>1.87 ± 0.4</td>
<td>0.06 ± 0.02</td>
<td>3.02 ± 0.74</td>
<td>2.6 ± 0.77</td>
</tr>
</tbody>
</table>

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individuals were included in their study, the total amounts of IL-1β, IL-4, IL-6 and IL-8 were measured in a total of 400 samples using commercially available enzyme-linked immunosorbent assays. Significant interactions between "EOP" and "smoking" were present for total amounts of IL-1beta and IL-4. IL-1beta, IL-6 and IL-8 showed significant main effects with healthy smokers and healthy non-smokers, respectively. They concluded that smoking influences host-related factors including cytokine network [30], evaluated association between passive smoking and salivary markers related to periodontitis among 273 workers using enzyme-linked immunosorbent assay. They found IL-1beta was elevated significantly in passive smokers relative to non-smokers. Similarly, in our study the IL-1β concentrations were found to be higher in the gingival crevicular fluid from periodontitis patients than in that from gingivitis patients; their result suggest that there is an association between severity of periodontal disease and levels of IL-1. Miller et al. [26] conducted a study to determine if salivary biomarkers specific for three aspects of periodontitis: inflammation, collagen degradation and bone turnover, correlated with clinical features of periodontal disease. They assessed the relationship between clinical parameters of periodontal disease and the levels of IL-1β, matrix metalloproteinase (MMP)-8, and osteoprotegerin (OPG) in whole saliva. They reported that the mean levels of IL-1β and MMP-8 in saliva were significantly higher in periodontitis subjects than in periodontally healthy individuals and that smoking causes an increased expression of IL-1β tissues which induce tissue destruction, bone resorption and production of matrix metalloproteinases and prostaglandin E2 [31] and consequently, destruction of periodontal tissue. An interested finding of this study is the significant correlation between PI, BOP, CAL, Bone loss and IL-1β that has resulted in a strong association with periodontitis. Similar finding was observed by Miller et al. [26].

### References
