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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.

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. Smoker subjects were further analysed about the number and the duration of the habit. Furthermore, smoking status of current smokers was determined based on the consumption; minimum of 10 cigarettes/day for not less than 2 years [10]. Smokers were divided into sub groups

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as light smokers and heavy smokers based on this equation Pack years=(No. of cigarette smokes per day×No. of years smokes)/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 base of the probing pocket depth [11], by using William's graduated periodontal probe. When CEJ was obliterated by the gingival margin, the CAL was measured as the following: 1-measuring the distance in millimetres from the free gingival margin (FGM) to the cementoenamel junction (CEJ). 2-mesuring the distance from FGM to the base of the pocket each site. 3-the attachment loss was obtained by subtracting the first measurement from the second one [17]. In some cases when there was gingival recession, attachment loss was measured by adding the distance from gingival margin to the CEJ to the probing depth, the level of the CEJ could be determined by feeling it with the probe. In some rare situations where the CEJ was totally obliterated by, full crown coverage, filling (disto-occlusal, mesio-oclusal, or mesio-occluso-distal), badly carious, in such situation, the tooth was excluded. The CAL for each patient was measured by summation of CAL measurement of each surface that must have CAL divided by their number. Four radiographs were taken for each subject including two bitewing radiographs (for right and left posterior tooth) and two periapical radiographs (for upper and lower anterior teeth) using paralleling technique. X-ray viewer and digital

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 (IL1 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=N/1+N (e)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 (IL1B, 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

Age group	IL1 β (pg/ml)	P value	PI M ± SD	P value	BOP M ± SD	P value	CAL (mm) M ± SD	P value	Bone loss M ± SD	P value
25-29	258.3 ± 162.9		1.1 ± 0.7		0.08 ± 0.10		1.2 ± 1.3		1 ± 1.1	
30-34	243.2 ± 194.6	0.390	1.2 ± 0.7	0.884	0.07 ± 0.10	0.867	1.2 ± 1.4	0.475	1 ± 1.2	0.418
35-40	309.1 ± 205.1		1.1 ± 0.8		0.09 ± 0.13		1.6 ± 1.6		1.4 ± 1.5	

Table 1: Explores different ages of studied groups in relation to periodontal measures.

Code	IL1 β [*] (pg/ml)	PI[*] M ± SD	BOP[*] M ± SD	CAL(mm)[*] M ± SD	Bone loss[*] M ± SD
Non-smoker Healthy	81.5 ± 51	0.44 ± 0.4	0.04 ± 0.10	0.0 ± 0.0	0.0 ± 0.0
Smoker healthy	124 ± 18.9	0.64 ± 0.4	0.01 ± 0.0	0.0 ± 0.0	0.00 ± 0.00
Non-smoker periodontitis	357 ± 61.7	1.72 ± 0.3	0.22 ± 0.1	2.5 ± 0.78	2.14 ± 0.76
Smoker periodontitis	525.8 ± 87.1	1.87 ± 0.4	0.06 ± 0.02	3.02 ± 0.74	2.6 ± 0.77

Table 2: Mean ± SD among studied group. [*]Indicates statistically significant result $p<0.05$.

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).

The PI between light and heavy smokers with periodontitis was 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 0.555, respectively); however, the association was not statistically significant (p ≥ 0.005). 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 ≥ 0.005). 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 IL1β than light smoker with periodontitis (335.14, 314.20, respectively), however, the association was not statistically significant (p value ≥ 0.05) (Table 4). In healthy persons, nearly comparable mean value of IL1β 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).

Discussion

In this study PI of non-smoker and smoker periodontitis were reported statistically highly significant than PI of non-smoker and smoker healthy individuals. While PI between smoker and non-smoker persons with periodontitis was not significant, other investigations have shown little difference in the level of plaque accumulation, comparing smokers with non-smokers. Calsina et al. [5] in their study on effects of smoking on periodontal tissues; they found that among cases plaque index did not show differences between smokers and non-smokers. On the other hand, Scabbia et al. [20] in their study showed smoker persons had significantly more plaque than non-smoker persons, the possible explanation for this difference may be due to the fact that the PI is depend on oral hygiene measurement or due to difference in methodology to measure the amount of plaque using disclosing agents [20].

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

groups	IL1 β (pg/ml)	P value	PI M ± SD	P value	BOP M ± SD	P value	CAL (mm) M ± SD	P value	Bone loss M ± SD	P value
Non-smoker periodontitis	357 ± 61.7	0.000	1.72 ± 0.3	0.305	0.22 ± 0.1	0.000	2.5 ± 0.78	0.020	2.14 ± 0.76	0.035
Smoker periodontitis	525.8 ± 87.1		1.87 ± 0.4		0.06 ± 0.02		3.02 ± 0.74		2.6 ± 0.77	

Table 3: Mean ± SD among periodontitis group.

Smoking status among smokers with periodontitis	IL1 β (pg/ml)	P value	PI M ± SD	P value	BOP M ± SD	P value	CAL (mm) M ± SD	P value	Bone loss M ± SD	P value
Light smoker	314.20 ± 196.93	0.45 6	1.93 ± 0.80	0.760	0.711 ± 0.34	0.211	2.7 ± 0.56	0.231	2.4 ± 0.58	0.21
Heavy smoker	335.14 ± 227.69		1.81 ± 0.73		0.555 ± 0.18		3.02 ± 0.84		2.8 ± 0.87	

Table 4: Mean ± SD among smoker periodontitis group.

Smoking status among smokers healthy group	IL1 β (pg/ml)	P value	PI M ± SD	P value	BOP M ± SD	P value	CAL (mm) M ± SD	P value	Bone loss M ± SD	P value
Healthy light smoker	126 ± 25.8	0.65	0.76 ± 0.31	0.000	0.01 ± 0.003	-	00 ± 00	-	00 ± 00	-
Healthy heavy smoker	122 ± 20.8		0.53 ± 0.24		0.01 ± 0.003		00 ± 00		00 ± 00	

Table 5: Mean ± SD among smoker periodontitis group.

		PI	BOP	CAL	Bone loss
IL-1 β	r	0.773	0.335	0.941	0.939
	p	0.00	0.00	0.00	0.00

Correlation is significant at the level 0.01

Table 6: Pearson correlation coefficients (r) with (P) values between IL1B and clinical parameters.

were 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-1 β, 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 controls. On the contrary, Teles et al. [27], in their study on 118 subjects 74 chronic periodontitis and 44 periodontally healthy individuals and the levels of GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ and TNF-α measured in whole saliva using a multiplexed bead immunoassay (Luminex). There were no statistically significant differences between groups for any of the cytokines and demonstrated salivary concentrations of IL-1β were not differ between subjects with chronic periodontitis and periodontally healthy subjects. This differences could be explained due to differences in methods of saliva collection (stimulated or unstimulated), processing (speed and time of centrifugation), storage (time, temperature and addition or not of protease inhibitors) and in the methodology used for the quantification of the biomarkers (ELISA versus Luminex) [27]; Compared with ELISA, Luminex have a number of advantages including; high throughput multiplex analysis; less sample volume needed, efficiency in terms of time and cost, ability to evaluate the levels of one given inflammatory molecule in the context of multiple others; ability to perform repeated measures of the same cytokine panels in the same subjects under the same experimental assay condition; ability to reliably detect different proteins across a broad dynamic range of concentrations [28]. Kamma et al. [29] evaluate the influence of cigarette smoking on the gingival crevicular fluid (GCF) levels of interleukin (IL)-1beta, IL-4, IL-6 and IL-8 among early onset periodontitis (EOP) patients and in healthy controls (H); sixty-five EOP and 35 periodontally healthy

individuals were included in their study, the total amounts of IL-1beta, 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 stratified by smoking, where the IL-1β concentration was higher in smoker healthy subjects and smoker subjects with periodontitis than in non-smoker healthy and non-smoker periodontitis which verified the interaction between salivary IL-1β and smoking. Possible explanation is 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].

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