Assessment of Interleukin-1β in Saliva of Smoker and Non-smoker Patients with Chronic Periodontitis

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Background and objectives: Smoking is considered a significant risk factor in periodontitis. The pathogenesis of periodontal disease may be affected by alterations of the inflammatory response by smoke. Interleukin-1 (IL-1β), an effective pro-inflammatory cytokine have been associated with the immunopathology of periodontitis. The aim of this study was to assess the levels of IL-1β in saliva from smokers and non-smokers with chronic periodontitis and periodontally healthy controls.

Materials and methods: Gingival index, Plaque index, probing pocket depth, clinical attachment level, bleeding on probing were assessed in 84 subjects who were equally divided in to three groups. group I periodontally healthy non-smoking subjects ; group II non-smokers with chronic periodontitis; group III smokers with chronic periodontitis; Enzyme Linked Immunosorbent Assay (ELISA) test was used for quantification of Interleukin (IL)- 1β in the saliva samples. Statistical analysis were performed with SPSS version 22.0.

Results: Interleukin-1β level in saliva was significantly higher in smoker than in nonsmokers with chronic periodontitis and the controls (P< 0.001). In subjects with chronic periodontitis, a significant correlation existed between CAL and IL-β (r= 0.313, p= 0.052) among non-smokers; there was also a highly significant positive correlation between, CAL and IL-β (r= 0.310, p= 0.051), BOP and IL-β (r= 0.333, p= 0.05) among smokers.

Conclusion: Salivary IL-1β level were significantly elevated in periodontitis patients. Smokers with chronic periodontitis exhibited a highly significant increase in salivary IL-1β levels. Hence, this reflects the impact of smoking on immune response and its role in the pathogenesis of periodontal disease.

Keywords: Chronic periodontitis, Saliva, Interleukin-1β, smoking

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Introduction

Chronic periodontitis is a bacterial-induced chronic inflammation within the structures that supportive tissue of teeth, resulting the progressive attachment and bone loss. [1] The onset and progression of periodontitis is due to an imbalance of the interaction between bacterial pathogens and host immunity. [2]

Host immunity is greatly influenced by both genetic susceptibility and environmental risk factors for periodontitis. Host behaviour, such as oral hygiene habits or smoking, also influence the course of the disease. [3] Although bacterial infection is the primary cause in triggering periodontal disease, subsequent progression and disease severity depends on the production of the host mediators in response to bacteria and it's metabolic products. [4] The presence of periodontal pathogens, such as Porphyromonas gingivalis, and Fusobacterium nucleatum has been found to be clearly culprits in periodontal destruction and are highly associated with periodontitis development. [2,5] The continuous challenge of host immune and resident cells by periodontal pathogens and their virulence factors results in a complex net-

work of pro-and anti-inflammatory cytokines performing in the inflamed periodontal tissues. These host mediators directly or indirectly assist in periodontal tissue destruction and predominantly in bone resorption. [6] A cytokine network, regulates the relations between periodontal pathogens and inflammatory process, being able to increase or suppress tissue reactions in periodontal pathogenesis. [7,8] Cytokines such as Interleukin (IL) IL-1B, IL-6, IL-8, IL-10 and TNF-α are the crucial cytokines involved in the host response of periodontal disease as mediators of tissue destruction, with further impact on the activity of immune cells. [9]

The Interleukin-1 (IL-1) existing in two active forms, IL-1α and IL-1β encoded by separate genes. Both are potent proinflammatory molecules and playing a central role in triggering and perpetuating the immune and inflammatory responses [8,10]. IL-1β is mostly produced by monocytes, macrophages and neutrophils and also by other cell types such as epithelial cells and fibroblasts. IL-1β increase expression of (Intra Cellular Adhesion Molecule) ICAM and secretion of chemokines. It is Considered as a critical factor of tissue destruction due to its pro-inflammatory and bone resorptive properties. It has a significant role in adaptive immunity, regulates antigen presenting cells and has revealed to improve antigens mediated of T-cells. [11,12] It is also mediate in cell proliferation, differentiation, and apoptosis in the pathophysiology of periodontitis. [13] The levels of pro-inflammatory cytokine IL-1β has been found to be significantly increased in the periodontal tissues and GCF from diseased sites, compared with healthy sites and are thought to be a critical determinant of periodontitis outcome. [14] Smoking is considered to be one of the most significant environmental risk factors, which is closely related not only with the risk but also the prognosis of periodontitis. [15] Smoking changes the host response through a plethora of changes that include vascular function, antibody production, activities of neutrophil/monocyte, and lastly influencing cytokine and inflammatory mediator release, and thus reveals a negative impact on the periodontium. [16] In this context, smoking acts as a natural and valued model for studying the pathogenesis of periodontitis. Variations in periodontitis incidence and severity between smokers and non-smokers have been well recognized. [17] More clinical attachment and bone loss have been observed among smokers than among nonsmokers. [18] The hypothesis to be confirmed was that whether there is any association between salivary IL-1β levels and clinical findings in smokers and non-smokers and to assess usefulness of IL-1β for diagnosis of periodontal severity.

Materials and methods

Study population. Study subjects were randomly selected from the Outpatient Department of Periodontology, College of Dentistry/ Hawler Medical University from January to May 2018. Ethical clearance for the study was obtained from the College's ethical committee, signed consent forms were obtained from all participants before conducting the study. The present study compromised a case-controlled study design. A total of eighty four male subjects (n=84; age ranging from 30-55 years; mean age of 37.5) were divided into three groups: Group I- 28 healthy individuals with no clinical and radiographic manifestations of periodontal disease; Group II- 28 non-smokers with chronic periodontitis; and Group III- 28 smokers (based on the self-reported smoking status) with chronic periodontitis.

Selection criteria for the subjects, Inclusion criteria. Presence of good general health and clinical diagnosis of periodontal disease (periodontally diseased patients included individuals with a probing pocket depth ≥4 mm and clinical attachment loss of ≥ 2 mm (> 30% affected sites). Periodontal diagnosis was assessed according to the classification of the American Academy of Periodontology. [19] The control group consisted of individuals without a history of periodontal disease and attachment loss, as well as with probing depth (PD ≤ 3mm and bleeding index (BI) simplified < 20% to exclude the presence of gingivitis.

Exclusion criteria. Subjects had received periodontal therapy in the last 6 months be-

fore sampling and recording, administration of systemic antibiotics in the last 6 months, and regular use of anti-inflammatory medications in the 6 months., subjects with chronic systematic disease such as: diabetes mellitus, hepatitis, rheumatoid arthritis, cardiovascular disease), tumors, alcoholism, and individual with less than twenty teeth were excluded from this research

Patient selection. Grouping of smokers and nonsmokers was adopted from the study by Tymkiw et al., [20] Smokers were classified and enrolled in this study if they regularly smoked ≥ 10 cigarettes per day, and nonsmokers were classified as not having smoked one hundred or more cigarettes in their lifetime.

Collection of saliva samples and analysis. Un-stimulated whole expectorated saliva was collected from each subjects according to the method described by Navazesh. [21] The subjects were refrained from eating, drinking, gum chewing, oral hygiene measures (flossing, brushing, and mouth rinses) for at least 2hours prior to the sampling. The subjects were asked to rinse their mouth with distilled water thoroughly to remove any food debris, tilt their head foreword and then expectorate at least 3 mL of un-stimulated whole saliva into a 5mL sterile plastic tubes for 5 min at 9-11 AM before doing periodontal examination. Collected samples were placed on ice pack immediately, then transported to the laboratory and centrifuged at 3000 rpm for 10 minutes. The obtained supernatant was placed into sterile Eppendorf vials and kept at -20°C till the time of the assay. Salivary IL-1β levels were measured with an ELISA kit using Human Interleukine-1 β (IL- β) ELISA Kit provide by REF (Catalog # DRE 10292). The test was run by an expert, blinded to the clinical results as per manufacturer's instructions. The calorimetric reaction in the wells was recognized by an ELISA reader with an optical density value set at 450nm. The concentration of IL- β was reported as nanograms per liter (ng/L) of the sample. The standard range was 1- 20 ng/L

Clinical periodontal examination

Clinical periodontal examinations of all participants were done on dental chair using periodontal probe (UNC probe-15, Hu-Friedy, Chicago, IL, UAS) by a single experienced examiner after collection of saliva sample. The clinical parameters assessed for the study were Gingival index (GI), Plaque index (PI), Probing pocket depth (PPD), Clinical attachment level (CAL) and Bleeding on probing (BOP). The score of each index was derived by examining four sites (Mesial, distal, buccal and lingual) Panoramic radiographs were taken for each participants for the determination of alveolar bone loss.

Statistical analysis. The descriptive data were expressed as mean, standard deviation (Mean ± SD) from the sample for each study group. Mean values were compared among different study groups by Kruskal-Wallis one-way ANOVA test. Pairs –wise comparison was performed by Tukey's honest significant difference (HSD) post hoc procedures to determine the difference of the salivary IL-1β level between the groups. Spearman rank correlation analysis was done to assess the relationship between salivary IL-1β level with clinical parameters among the three groups. In this study, $P < 0.05$ was considered as the level of significance. The statistical software SPSS version 22 was used for the analysis of the data.

Results

A total of eighty four male subjects were examined for this study, with 28 participants in each group. The mean age of the healthy control group was 39.10 ± 9.62, that of non- smokers with CP was38.85 ± 9.55 years, and that of smokers with CP was 39.77 ± 9.30 years. There was no statistically significant difference in the mean age among the three study groups ($p> 0.05$).

The mean GI values among the smokers (1.29± 0.27) was significantly less than that of nonsmokers (2.28 ± 0.29; $p< 0.001$); whereas the mean PI, PPD, and CAL values were higher in smokers than nonsmokers with chronic periodontitis. All values were statistically significant ($P<0.001$). Among periodontitis subjects, the mean value of BOP was significantly higher among non-smokers with CP than to smokers with CP (58.47 ± 6.10, 56.34±5.50 respectively) ($p \geq 0.001$) (Table 1).

The mean of salivary IL-1β concentration

was (7.62± 0.87 ng/L) in smokers with CP, (5.58 ± 0.79 ng/L) in non-smoker with CP and (1.60 ± 0.34 ng/L) in healthy control group. Among periodontitis subjects, a statistically highly significant difference ($P < 0.0001$) was found in the salivary IL-1β concentration between smokers and non-smokers ($P < 0.0001$), and statistically high significant difference ($P < 0.0001$) was found between smoker with CP and healthy control group and between non-smokers with CP and healthy control group (Table 1 and Graph 1). Moreover, with respect to salivary mean levels of IL-1β, a pair-wise comparison (Post Hoc test) between the groups also showed a highly statistically significant differences ($P < 0.0001$), as clearly show in (Table 2).

The correlation between salivary IL-1β levels and clinical parameters (GI, PI, PPD, CAL and BOP) among the study groups was analysed. There were no statistically significant correlation between the salivary IL-1β and clinical parameters in the control group ($p> 0.05$). Spearman correlation co-efficient analysis, shows positive statistically significant correlation between CAL and salivary IL-1β ($r=0.313$, $p= 0.052$) among Non-smokers with CP. A highly significant positive correlation was observed between CAL and IL-1β ($r=0.310$, $p= 0.051$), BOP and IL-1β ($r=0.333$, $p= 0.050$) among smokers with CP (Table 3).

Discussion

In this study, we analysed the salivary levels of IL-1β in smokers and non-smokers with CP patients in an attempt to clarify possible mechanisms of the damaging effects of smoking on periodontal tissues. Tobacco smokers are stated to exhibit an

Table 1: Comparison of mean values of clinical parameters and IL-1β concentrations among the three study groups.

Groups differences				
F value	P value ANOVA	Groups		
Control N=28 (Mean±SD)	Nonsmoker N=28 (Mean±SD)	Smoker N=28 (Mean±SD)		
Clinical Parameters/ IL-1β concentration				
0.00	345.11	1.29±.27	2.28 ± 0.29	0.46 ± 0.23 GI
0.00	129.89	1.90±.63	1.37 ± 0.27	0.33 ± 0.24 PI
0.00	143.44	5.30±0.53	4.70 ± 0.68	1.79 ± 0.65 PPD (mm)
0.00	491.82	5.30±0.75	4.24 ± 0.73	0.00 ± 0.00 CAL (mm)
0.00	1308.70	56.34±5.50	58.47 ± 6.10	0.00 ± 0.000 BOP (%)
0.00	331.44	7.62±.87	5.58 ± 0.79	1.60 ± 0.34 IL-1β (ng/L)

GI; Gingival Index,PI; Plaque index, PPD; Probing pocket depth, CAL; Clinical attachment loss, BOP; Bleeding on probing,IL-1β ; Interleukine-1 Beta, Values are mean± SD; Standard deviation, F values is based on One-way ANOVA test; P-values ≤ 0.05 is considered to be statistically significant

Table 2: Comparison of the mean values of IL-1β (ng/L) among the three study groups by post-hoc (TukeyHSD) test.

Dependent variable	Group(J)	Significant	Standard error	Mean differences (I-J)	95% Confidence interval	
					Lower bound	Upper bound
IL-1β (ng/L) (Saliva)	N(CP) vs C	0.18	-3.98 ± .0.93	-4.34	-3.63	0.00
	S(CP) vs C	0.19	-6.025 ± 0.93	-6.39	-5.67	0.00
	N(CP) vs S(CP)	0.23	-2.05 ± 1.22	-2.53	-1.57	0.00

IL-1β ; Interleukine-1 Beta, CP; Chronic periodontitis; NS(CP) = Non-smoker chronic periodontitis; S(CP) = Smoker chronic periodontitis; C= Control group **; p < 0.001, Indicates Highly significant; HSD= Honest significant difference, Pair-wise comparison is by Tukey's post hoc procedures.

Table 3: Correlation coefficient of IL-β with clinical parameter

Clinical Parameter	Interleukin-1 beta (IL-1β)	r	P-value
Control N=28	GI	0.214	0.24
	PI	- 0.109	0.57
	PPD	- 0.132	0.53
	CAL	----	----
	BOP	----	----
Non-smoker with CP N=28	GI	0.134	0.48
	PI	- 0.114	0.56
	PPD	- 0.204	0.29
	CAL	0.459	0.01*
	BOP	0.166	0.39
Smokers with CP N=28	GI	-0.057	0.91
	PI	0.191	0.33
	PPD	-0.143	0.51
	CAL	0.786	0.05*
	BOP	0.345	0.03*

r= Spearman rank correlation coefficient

increased susceptibility for development and progression of periodontal diseases. [22] Smokers tend to display excess production of inflammatory mediators, such as IL-1β, IL-6, IL-8, TNF-α and increased neutrophil derived proteolytic enzymes which could promote to periodontitis progression and periodontal tissue destruction. IL-1β is a multifunctional cytokine that regulates various cellular and tissue functions. [23] Accordingly, we hypothesized that the significantly increased susceptibility to tobacco smokers to the development and progression of periodontitis may be due, in part, to tobacco-induced increases in salivary levels of IL-1β. The present study showed the gingival index (GI) was significantly higher in non-smokers with chronic periodontitis than in smokers with periodontitis ($P < 0.001$). Studies by [24,25] showed similar results. This supports the conception that smokers mostly present with reduced gingival inflammation compared to non-smokers because smoking has a strong, chronic, dose-dependent suppressive effect on gingival inflammation. [26]

In this study, mean value of PI was higher among non-smoker and smoker CP patients than non-smoker healthy group. This association was statistically highly significant. Scabbia et al., [27] in their study showed smoker persons had significantly more plaque than non-smoker persons. In addition to that Gaphor et al., [28], showed that PI of non-smoker and smoker CP patients were statistically highly significant than PI of non-smoker and smoker healthy individuals. On the contrary, Calsina et al., [29] they observed that among cases plaque index did not show differences between smokers and non-smokers, the possible explanation for this variance may be due to the fact that the PI is depending on oral hygiene measurement or due to difference in methodology to measure the amount of plaque using disclosing agents. [27]

Our study revealed that smokers with CP had significantly increased mean values of PPD and CAL compared to non-smokers with CP. Our findings agree with the studies by Calsina et al ., [29] and Patel et al., [30] who also reported the dose relationship between the effect of cigarette consumption and periodontal attachment loss. Similarly Jenifer et al., [25] and Mahuca et al., [31] in their study, they reported greater probing depth and attachment loss were in smokers. The possible explanation is the cumulative effect of smoking on periodontal microbial, immune impairment and cytokine up-regulation. Literature is alienates with suggestion that support the smokers are at a higher risk of having periodontitis with higher attachment loss as compared with non-smokers. [28,30]

BOP is a value for the initial diagnosis and prevention of periodontal disease and used as a predictor of periodontal disruption in retrospective and prospective clinical studies. [32] In this study, mean value of bleeding on probing index was higher in non-smoker than smoker periodontitis. This association was statistically highly significant. This is in agreement with findings of other studies [24,28,29,31] who also reported higher bleeding indices in non-smokers with CP than smokers with CP. This finding could be elucidated due to nicotine convinced vasoconstriction in smoker's gingiva as well as heavy gingival keratinization in smokers. [33]

Regarding IL-1β Concentration in saliva, the results of the current study showed significantly increased IL-1β concentrations in the saliva of smokers with CP, compared to non-smokers with CP and healthy controls ($P \geq 0.01$), which confirmed the interaction between salivary IL-1β and smoking. Similarly to our findings, Gaphor et al.,[28] who reported that the mean value of IL-1β in saliva was significantly higher among smokers and non-smokers with CP as compared with smoker and non-smoker healthy subjects; additionally they concluded significantly higher salivary levels of IL-1β among smokers with CP. This relative increase in IL-1β in smokers is consistent with a previous study by Patel et al.,[30] who reported higher GCF levels of IL-1β among smokers. Al-Ghurabi et al., [24] showed that mean values of IL-1β, IL-8 and IL-17 in serum were also significantly higher in smokers than non-smokers CP patients. A possible explanation is that smoking causes higher expression of IL-1β tissues which encourage tissue destruction, bone resorption, production of matrix metalloproteinases and prostaglandin E2 and consequently,

destruction of periodontal tissue. [34]This also could be explained by the fact that nicotine, an active content of smoke, impairs the gingival blood flow with the vasoconstrictive properties and creates an anaerobic environment conductive for the growth of periodontal bacteria. [35] An increase in these anaerobes enhances greater stimulation of the gingival monocytes and macrophages, resulting in an increased production of IL-1β [34,35]. Moreover, the oral keratinocytes are quite sensitive to cigarette smoke and reac with an increased production of inflammatory mediators such as PGE2 and IL-1. contributing to periodontal destruction[36] Altogether, results of these studies suggest that there is an association between severity of periodontal disease and levels of IL-1β. On the other hand, these results lead to uncovering the basis for the etiological role of cigarette smoking in periodontitis.

In contrast to our findings, Rawlinson et al., [37] who reported lower GCF levels of IL-1β among smokers vs non-smokers, suggested that production of pro-inflammatory biomarkers is depressed in smokers, but these mediators are still present at concentrations capable of pathogenesis. Moeintaghavi et al., [38] they reported that smoker periodontitis showed lower IL-1b gene expression than non-smoker periodontitis.

In present study the correlation between clinical periodontal parameters and salivary IL-1β levels among three groups was analysed. There was no significant correlation within the control group, when salivary IL-1β level was correlated with the clinical parameters like GI,PI, PPD,CAL, and BOP. An interested finding of this study was that, in smokers with CP, there was a positive significant correlation between CAL, BOP and salivary IL -1β ($p< 0.05$). This indicates that there is a correlation between the increase of CAL and BOP values and the increase of salivary IL-1β level. In non-smokers with CP, it was found a positive significant correlation between the CAL and salivary IL-1β ($p< 0.05$). This finding suggests that IL-1β is highly related to the inflammatory condition of periodontium. Thus the presence of elevated levels of IL-1β in saliva of CP, along with the significant correlation with clinical assessments of periodontal tissue destruction, strongly suggests an important role of IL-1β in the pathogenesis of periodontal disease. This is in agreement with the findings of another study by Gaphor et al., [28] who reported a statistically significant correlation between PI,CAL, BOP and salivary IL-1βlevels that has resulted in a strong association with periodontitis. Similar findings was observed by Miller et al., [39] showed that when periodontitis is in the active state, the immune defence mechanism is activated and PMNs increase their release of IL-1β.

Conclusion

Smokers with CP exhibited higher levels of salivary IL-1β compared to non-smokers with CP, indicates that IL-1β may be a significant factor that modulates the increased periodontal destruction in smokers. This study concludes that salivary levels of IL-1β could be a very significant marker for periodontal breakdown in smokers.

Conflicts of interest

The authors reported no conflict of interest.

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