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Unstimulated Whole Salivary (SLPI & IL-1RA) Levels in Relation to the Cigarette Smoking at Periodontitis Patients

Rehab Faisal Ahmed, Wedad Farhan Jaber

ABSTRACT

Background: **Gingivitis/periodontitis is one of the widespread chronic diseases affecting the supporting structures of teeth. Tobacco smoking is a significant risk factor for periodontitis and may affect the vasculature, the humeral immune system and the inflammatory system. The aim of the present study was the determination of salivary biomarkers including Secretory Leukocyte Peptidase Inhibitor (SLPI) and Interleukin - 1Receptor Antagonist (IL-1RA) at periodontitis subjects with and without cigarette smoking habit.**

Materials and Methods: **A total of 60 subjects [34 (56.7%) females and 26 (43.3%) males] with periodontitis were examined, the age range was between (18-36) years. The subjects were divided into a group of 40 non-smokers and another group of 20 smokers. Unstimulated whole saliva was collected from each subject; gingival index (GI) and periodontal disease index were recorded during clinical visit. Levels of salivary SLPI and IL- 1RA were measured by using ELISA immunoassay analysis.**

Results: **The median attachment loss and pocket depth were significantly different (P = 0.019, P = 0.038 respectively) among periodontitis cases with smoking habit. The median loss of attachment and pocket depth were significantly higher in the smoking group (1.48 and 2.13 respectively) compared to the non-smoking group (0.42 and 1.42 respectively), whereas the median GI showed no significant difference (P = 0.28). The Salivary SLPI level was elevated in the smoking group, whereas the salivary IL-1RA level was decreased but this decreasing failed to reach statistical significant level (P = 0.39 and P = 0.97).**

Conclusion: **The median attachment loss and pocket depth increase at smokers in comparison to non-smokers subjects. Salivary biomarkers including SLPI and IL-1RA can be used as predictors for progression of periodontitis.**

KEY WORDS

smoking, Saliva, periodontal health status

INTRODUCTION

Microbial dental plaque is the first strongest risk factor for periodontal disease followed by cigarette smoking; these two factors usually impair the various aspects of acquired and innate immune responses. The oral fluids molecules, as well as molecules in serum or plasma have been investigated to provide a specific and sensitive marker for periodontal tissue destruction[1].

According to Pucher & Stewart[2], the periodontal disease is a group of chronic inflammatory disorders that are associated with damage of the periodontal attachment apparatus (cementum, collagen fibrils and a layer of calcified inter fibrillar matrix on the root surface of the tooth). In normal circumstances, the balance between host response and microbial virulence factors was present, when periodontitis occurs, this balance will be impaired. The environmental factor that has been proposed to interact with host cells and influence on the inflammatory processes is the smoking[3].

Unstimulated whole saliva is "the mixture of secretion which enters the mouth in the absence of exogenous stimuli such as tastants or chewing". In resting state, the submandibular gland is the main gland for saliva secretion, whereas parotid gland produces only (20%) followed by sublingual glands (8%)[4].

The pro inflammatory protein that arrests the function beta cells and develops apoptosis is interleukin-1. In normal state, the body secretes anti-inflammatory proteins (interleukin-1 receptor antagonist) which are characterized by having a protecting function for beta cell and inhibiting effect on interleukin-1 protein so it is used to protect the periodontium from the inflammatory effect of interleukin-1[5]. On the other hand, saliva contains a spectrum of different proteins that are characterized by having antimicrobial properties[6]. The SLPI has been suggested to be the main soluble factor in saliva which potently protects adherent monocytes and activated peripheral blood mononuclear cells against infections. It is secreted by neutrophils, macrophages and mucous membrane epithelial cell and it can regulate various factors responsible for causing inflammation[7].

MATERIALS AND METHODS

Sixty patients with periodontitis (from both genders with age range of 18-36 years) attended the dental clinic of Dentistry College/ (University of Baghdad) during a 4- months period from (March 2016 to June 2016). Exclusion criteria include: patients who took antimicrobial agent within 1 month, presence of diabetes mellitus or any other systemic diseases and pregnant women. Individuals who smoked at least 20 cigarettes per day for 5 years were considered smokers. The subjects

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Department of Oral Diagnosis, College of Dentistry, University of Anbar
Iraq
Correspondence to: Wedad Farhan Jabber
(e-mail: widad.jabber@yahoo.com)

Table 1. Number & percentage of periodontitis subjects by selected variables (smoking habit, age, and gender)

Selected variables	Number	Percentage (%)
Smoking habit		
Non-smokers	40	66.7
Smokers	20	33.3
Age groups		
< 20 years	11	18.3
20-24 years	23	38.3
25-29 years	16	26.7
30 + years	10	16.7
Gender		
Male	26	43.3
Female	34	56.7

Table 2. Salivary and periodontal health parameters (mean/median) and Standard deviation among the study groups according to the smoking habit

Salivary and periodontal health parameters	Non-smokers' group	Smokers' group	Statistical analysis
	mean/median	mean/median	P-value
SLPI (ng/ml)	30.8(± 15.4)	35.2(± 10.5)	0.39[NS]**
IL-1RA (ng/ml)	16(± 9)	15.8(± 8)	0.97[NS]**
Attachment loss	0.42	1.48	0.019*
Pocket depth	1.42	2.13	0.038*
Gingival index	1.2	1.35	0.28[NS]**

* ($p < 0.05$) significant, ** ($p > 0.05$) non-significant

Table 3. Correlations of salivary parameters (SLPI & IL-1RA) and periodontal health status (GI, attachment loss & pocket depth) in the smokers' group

	Salivary parameters and periodontal health status	
	IL-1RA (ng/ml)	Pocket depth (mm)
SLPI (ng/ml)	r = 0.579 p < 0.001*	
GI	r = -0.262 p = 0.043**	r = 0.667 p < 0.001*
Attachment loss (mm)		r = 0.393 p = 0.002**

*($p < 0.01$) highly significant, ** ($p < 0.05$) significant

were divided into two groups: 40 non-smokers and 20 smokers, a female group of 34 (56.7%) and a male group of 26 (43.3%). Unstimulated whole saliva sample was collected in the morning by spitting method after asking the patient to rinse his mouth with water. Three to four ml of saliva was collected into a pre-labeled sterile container for 10 minutes. To remove any unwanted particles, the saliva was centrifuged at 3000 rpm and the supernatant has been aliquot into eppendrof tubes then the sample was stored in -70℃ pending analysis. An enzyme-linked immunosorbent assay (ELISA technique) was used to determine the biomarkers' concentration in saliva by using specific kit for SLPI & IL-1RA according to the instruction of each kit (BioAssay ELISA Kit, U.S.A.). The concentration of salivary sample was diluted by using phosphate buffer 150 fold; new concentration read from the standard curve should be multiplied by dilution factor. The gingival index (GI) and periodontal disease index[8] were all recorded for all participants.

Statistical analysis: The results were evaluated statistically by using the independent samples t-test and accepted as significant at $P < 0.05$ and as highly significant at $P < 0.01$.

RESULTS

The (mean ± SD) age in periodontitis individuals was (24.8 ± 5.4). Frequency distribution of the sample according to the gender, age and smoking habit were illustrated in Table 1.

The mean salivary SLPI level in Table 2 was higher among the smokers' group than among the non-smokers' group, but the result revealed that the difference was non-significant ($p = 0.39$). IL-1RA level was decreased at smokers in comparison to nonsmokers, but this difference was statistically not significant ($p = 0.97$). The median attachment loss and pocket depth were significantly lower in the non-smokers' group (0.42, 1.42 respectively) in comparison to the smokers' group (1.48, 2.13 respectively), also the median gingival index (GI) was decreased in the non-smokers group than in the smokers', but the result revealed no significant difference ($p = 0.28$).

The correlation of SLPI with IL-1RA was highly significant in positive direction ($r = 0.579$, $p < 0.001$). Salivary IL-1RA showed a statistically significant weak negative correlation with gingival index ($r = -0.262$, $p = 0.043$). The correlation between pocket depth and GI was highly significant in positive correlated ($r = 0.667$, $p < 0.001$), also the correlation of pocket depth with clinical attachment loss was significant in positive direction ($r = 0.393$, $p = 0.002$) (table 3).

DISCUSSION

The most common oral disease which is characterized by gingival inflammation and alveolar bone resorption is periodontitis[9]. The directly or indirectly deterioration of periodontal tissues occur due to the toxic products of tobacco smoking, especially the nicotine. A high-risk individual for periodontitis progression was heavy smoker and blood cotinine that related to smoking should be considered as significant risk markers for periodontal diseases prognosis[10].

According to the result of this study, there were higher SLPI and lower IL-1RA levels in the smokers' group in comparison to the non-smokers' group, but the difference was not significant and this may be related to the small number of patients. The reduced level of IL-1RA among the smokers' group can be related to the inflammation of gingival tissue and periodontium which occur directly due to the bacteria by releasing damaging proteolytic and hydrolytic enzymes to cause the diseases. Alternatively the bacteria inducts the dis-regulation response of the host which results from the interference with surrounding host cells[11]; while the high level of SLPI in saliva was related to periodontitis because the SLPI plays a critical role in the control of excessive tissue destruction and mediates wound healing[12]. This result agrees with the study by Cox *et al*.,[13] who reported that the SLPI level increased significantly in gingivitis and periodontitis.

Data of this study revealed that the subjects in the smokers' group have high median pocket depth and attachment in comparison to the non-smokers', usually the host response is affected by environmental factors (such as smoking) which is considered a risk factor for chronic periodontitis at adults and adolescents[14]. Several mechanisms of smoking are affecting the immune system and impair host response either systemically by increased neutrophils number in peripheral blood or impaired migration ability through capillary walls and response to the bacterial that are found sub-gingivally[15] or locally in GCF and saliva by changing the biomarker levels in the GCF[16]. This finding goes in the same direction with some studies[17] that showed a strong relationship between smoking and attachment loss, also with a study by Bouclin *et al*.,[18] who found that the cigarette smokers had significantly greater pocket depth than nonsmokers.

On other the hand, the median gingival index was higher in the smokers' group than in the non-smokers' group, but the differences did not reach the statistical significance level, this finding being attributed to the reduced manifestation of clinical symptoms of inflammation due to less vascularity in smokers compared to nonsmokers.

One cigarette smoking has been proposed to have the capacity to cause a severe drop in blood flow within the gingival tissues[19], vasoconstrictive attacks and impairment of revascularization that may contribute to disrupt the immune defense delay healing response and increase the

risk of periodontal disease[20]. This result agrees with the study by Maryam *et al*.,[21] who found that the vascularity was significantly higher at nonsmokers compared to smokers and in disagreement with Rahman *et al*.,[22] who reported greater vascularity at smokers compared to non-smokers, in contrast with studies[23] that showed no significant difference between vascularity of smokers and nonsmokers.

The correlation between gingival index and IL-1RA was significant in negative direction ($r = -0.262$, $p = 0.043$) (Table 3) . The interleukin -1 receptor antagonist belonging to the interelukin-1 family counter acts the inflammatory effects of both types of interleukin-1 (IL-1 alpha & IL-1beta) and plays an evident role in the prevention of many inflammatory diseases[24]. This finding was in agreement with the study by Arend[25] who found that the dis-regulation in the balance between IL-1 and IL-1RA can be considered one of the factors that are affecting the susceptibility to and the severity of various diseases, so in the gingival inflammation and periodontitis there is a high secretion of IL-1 from immune cell whereas the level of IL-1RA expression to counter this inflammation becomes decreased.

Moreover, the correlation between the SLPI and IL-1RA was highly significant in positive direction ($r = 0.579$, $p < 0.001$), both IL-1RA and SLPI have an inhibitory action against the bacteria and the relationship between them was observed in the inflammatory process[5]. When inflammatory disease occurs, the SLPI concentration is altered; SLPI accumulation in local environment may represent an intrinsic feedback mechanism to prevent harmful effect of inflammation because it has an inhibitory activity against bacteria, virus and fungi, so at patients with periodontitis when the SLPI level becomes lower in saliva and GCF, the bacteria in the mouth and dental plaque begin to dominate triggers enhanced immune responses and secreted the endotoxin by potential inflammatory effect through dental plaque which lead to non-resolving inflammatory processes[26]. On other hand the IL-1RA was decreased in saliva due to the highly level of salivary IL-1 which is considered a pro-inflammatory cytokine that is secreted from immune cells when there is gingival inflammation and periodontitis[25].

In the current study there is a positive highly significant correlation between pocket depth and gingival index and a positive significant correlation with attachment loss in the smokers group. This could be explained by the destruction of periodontal tissues that is generated from an aggressive immune response, which leads to produce an inflammatory cytokines against the microorganism[27], in addition to that, the etiology of periodontitis has been linked to many factors; the first one is poor oral hygiene, which leads to dental plaque formation[28]. Moreover, habits such as smoking, tobacco and drug abuse also contribute to periodontitis progression[29]. This result agrees with several studies[30] which reported that there was a rapid and strong influence of smoking on the gingival health and periodontitis prevalence was found to be elevated among the smokers group.

CONCLUSION

In conclusion, smoking has a detrimental effect on cytokines as well as it decreased the level of IL-1RA and increased the SLPI level in saliva and gingival crevicular fluid. The most important risk factor and the relationship between smoking and periodontal diseases suggest that there is a positive effect of smoking on biochemical and clinical signs of gingivitis and periodontitis.

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