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Assessment of Salivary Macrophage Inflammatory Protein-1 Alpha Level in Different Stages of Periodontitis

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ABSTRACT

Introduction: Microbial plaque causes initiation of periodontal diseases, and it induces an inflammatory host immune response. Macrophage Inflammatory Protein-1 alpha (MIP-1α) is a chemokine that is thought to have a role in periodontitis by activating osteoclasts and mediating T helper-1 cytokines that promote destruction of tissues.

Aims: This study's aim is to evaluate salivary levels of MIP-1α in different stages (I-III) of periodontitis in comparison to healthy controls and to correlate their levels with clinical periodontal parameters.

Materials and methods: The sample population which consisted of (70) male and female participants was allocated into four groups: clinically healthy periodontium control group (n=10), stage I periodontitis group (n=20), stage II periodontitis group (n=20) and stage III periodontitis group (n=20). Collection of whole unstipulated salivary samples from all participants was carried out, followed by examination of clinical periodontal parameters (plaque index, probing pocket depth, bleeding on probing and clinical attachment level). Collected saliva was subjected to biochemical analysis using Enzyme Linked Immunosorbent Assay (ELISA) for detection of MIP-1α levels.

Results and discussion: Statistical analysis of clinical periodontal parameters revealed increased mean percentage of the clinical parameter with increased severity of periodontitis with significant difference. Statistical analysis of MIP-1α revealed increase in MIP-1α level with increased severity of periodontitis with significant difference. These results could be attributable to the role MIP-1α has in inflammatory immune response and osteoclast activation.

Conclusion: The discoveries of the study indicate that salivary MIP-1α could be used to differentiate different stages of periodontitis.

Key words: Periodontitis, Saliva, Chemokine's, MIP-1α

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INTRODUCTION

Periodontitis is a set of serious inflammatory conditions which results in the destruction of supporting teeth structures [1]. This destructive process occurs by two mechanisms: direct tissue damage from products of plaque bacteria, and indirect damage caused by bacterial stimulation of immune and inflammatory host responses [2]. Complex interactions between cells, extracellular matrix and circulating cytokines are involved in the host response [3]. Cytokines production stimulates inflammatory events that play a significant part in recruitment of leukocytes and might modulate osteoclast formation [4]. Elevated levels of circulating molecules have been identified in whole saliva and gingival crevicular fluid of periodontal disease patients [5].

The chemokine MIP-1α is amongst the mediators possibly involved in leukocyte migration to periodontal tissues. MIP-1α has been thought to have an essential part in periodontitis by activating osteoclasts and mediating Th-1 cytokines that promote destruction of tissues [6]. MIP-1α can as well stimulate osteoclastogenesis and induce osteoclast formation [7]. Bone resorption by MIP-1α is mainly through its CCR1 receptor. This receptor is expressed primarily by osteoclast cells.

In dental practice, clinical parameters are employed for periodontal disease, however numerous problems arise with using the clinical criteria for addressing the public needs in determining the present disease status/ progression, and demanding a significant extent of damage for these parameters to document disease. Therefore, a quick, simple and consistent technique of periodontal disease monitoring and assessment should deliver essential diagnostic information that speeds and develops treatment decisions and transfers the field a lot closer to more personalized diagnostics. Salivary analysis can serve as a beneficial tool in assessment of existing periodontal status, monitoring treatment response and

prediction of disease progression. Periodontal disease salivary biomarkers include host origin proteins (immunoglobulin's and enzymes), host cells, phenotypic markers, volatile compounds, hormones, bacteria and bacterial substances, and ions [8].

MATERIALS AND METHODS

The human sample involved (70) male and female subjects with age range of (30-60) years fit the criteria of the study. Sample collection was started from February 2021 until August 2021 at the department of periodontics in the teaching hospital of the college of dentistry, University of Baghdad.

The Inclusion criteria included patients with 20 teeth minimum, systemically healthy patients, patients who did not take antibiotic and anti-inflammatory drugs in the last three months and patients with age range between 30 to 60 years old. Exclusion criteria included patients who have undergone or currently under extensive periodontal treatment, a course of anti-inflammatory or antimicrobial therapy during the last 3 months, smoking or alcohol drinking, patients with chronic systemic disease, immunocompromised patients, pregnant, on contraceptive pills and lactating women, patients with diseases of soft and hard palate and mucosa such as white and red lesions and ulcers, patients wearing orthodontic appliances, removable dentures, implant, crown and bridge, patients with active COVID-19 virus infection.

The study participants were divided into these four groups:

- **Group A clinically healthy periodontium (control):** consisted of 10 subjects who had no probing attachment loss, probing pocket depths ≤ 3 mm, bleeding on probing <10% and no radiological bone loss.
- **Group B stage I early/mild periodontitis:** consisted of 20 subjects in which interproximal bone loss <15% or 1-2 mm clinical attachment loss.
- **Group C stage II moderate periodontitis:** consisted of 20 subjects in which interproximal bone loss involved coronal root third or 3-4 mm clinical attachment loss.
- **Group D stage III severe periodontitis:** consisted of 20 subjects in which interproximal bone loss involved middle third of root or ≥ 5 mm clinical attachment loss.

The samples of unstipulated whole saliva were collected from participants of all four groups under standard conditions [9]. Subjects had been asked to not drink or eat for 1-2 hours prior to saliva collection and to cleanse their mouths thoroughly with water to get rid of any debris or contaminating material before saliva collection.

The samples were put in a small cooling box after collection to stop bacterial growth. The tube was labelled with the number of the subject corresponding to that written previously on the case sheet. The samples were centrifuged at 2500 rpm for 20 minutes by centrifuge machine (80-1 electronic centrifuge, China) to separate the clear supernatant and aspirating it with a micropipette into Eppendorf tubes to be stored at -80°C freezers until the day of analysis. All samples were thawed to room temperature before being analysed.

After salivary sample collection, all the clinical periodontal parameters (PLI, BOP, PPD and CAL) were measured by a periodontal probe (the university of Michigan O probe with Williams marking at 1,2,3,5,7,8,9 and 10 mm). Six surfaces (four for plaque assessment) were examined from all teeth except the wisdom teeth.

The biochemical analysis of salivary MIP-1α was done using a kit manufactured by Shanghai Yehua Biological Technology Co, Ltd using ELISA technique

Statistical analysis was completed utilizing SPSS21 software. Mean and SD, Levine's test, *Chi square,* ANOVA test, Mann-Whitney test, Kruskal-Wallis test and spearman correlation coefficient (r) were used. Level of significance was: Not significant P>0.05, Significant P<0.05 and highly significant at p<0.01.

RESULTS

Outcomes of the study showed that the lowest mean values of (PLI, BOP, PPD and CAL) were found in the control healthy periodontium group and their values increased with increased severity of periodontitis with significant difference, as indicated in Table 1. Table 2 demonstrates the descriptive statistics for MIP-1α analyzation among study groups. Findings show that the lowest mean value of MIP-1α was found in the control healthy periodontium group and it increased with stages of periodontitis' progression with significant difference among groups. Regarding pearson's correlation coefficient between MIP-1α and clinical periodontal parameters in study groups, there was a significant moderate positive correlation of MIP-1α with PLI only in stage III periodontitis group as indicated in Table 3.

Table 1: Descriptive and statistical test of clinical periodontal parameters among groups using One Way ANOVA.

Groups	PLI	BOP	PPD	CAL
	Mean ± S.D.			
Control	57.330 ± 9.718	8.146 ± 1.363	1.228 ± 0.180	-
Stage I	78.805 ± 12.171	55.005 ± 17.749	3.635 ± 0.139	1.845 ± 0.204
Stage II	82.570 ± 9.568	64.255 ± 12.897	4.455 ± 0.267	2.669 ± 0.471

Stage III	85.407 ± 9.424	71.460 ± 16.847	5.405 ± 0.654	3.815 ± 0.602
P value	0	0	0	0
	Sig.	Sig.	Sig.	Sig.

Table 2: Descriptive and statistical test of MIP-1α (ng/mL) among groups using One Way ANOVA.

Groups	Mean ± SD	P value
Control	213.165 ± 35.169	0 sig.
Stage I	286.484 ± 46.735	
Stage II	330.225 ± 56.415	
Stage III	454.643 ± 120.659	

Table 3: Correlation of salivary MIP-1α with clinical periodontal parameters in study groups.

Groups	PLI		BOP		PPD		CAL	
	r	p value	r	p value	r	p value	r	p value
Control	-0.141	0.698	0.283	0.429	-0.071	0.846		
Stage I	0.128	0.59	0.338	0.145	-0.39	0.846	0.307	0.188
Stage II	0.138	0.561	0.271	0.249	0.051	0.846	0.266	0.257
Stage III	0.494	0.027	0.427	0.06	-0.204	0.846	-0.055	0.816

DISCUSSION

The findings of the present research have shown that the lowest mean percentage of PLI was found in the control healthy periodontium group and its values increased with increased level of periodontitis with significant difference. These findings could be because control subjects had healthy periodontium and they were performing good oral hygiene measures and good plaque control and also by the fact that the microbial biofilm is considered the primary and the major etiological factor responsible for initiation of periodontal disease [10]. These results agree with Khamees and Mohammad, 2012 [11] and with Saliem, 2016 [12]. Where they found that mean values of PLI were higher in severe group than other groups. Results of the lowest mean value of percentage of BOP was in the control healthy periodontium group and its level increased with increased stages of periodontitis with significant difference. These finding indicate the effect of plaque accumulation on blood circulation and the actual

pathophysiological process that happened more in inflamed tissues and the severity of bleeding and the ease of its provocation depend on the intensity of the inflammation [13]. These results agree with Saliem, 2016 and Ali and Mahmood, 2018 [14]. where they found that the percentage of bleeding sites for periodontitis groups was greater than that of the control healthy group.

PPD and CAL were the lowest in the control healthy periodontium group while it increased its level from Stage I to Stage III groups with significant difference. These could be due to increased bacterial invasion and the amount of plaque that resulted in the sulcular and junctional epithelium destruction and damage of surrounding alveolar bone in periodontitis. These results agree with Mousa, 2016 [15], Talib and Ahmed, 2016 [16] and Al-Rawi, et al. 2011 [17]. Where there were increasing in the values of CAL and PPD in periodontitis compared to the healthy subjects with a highly significant difference.

The biochemical analysis of MIP-1α results revealed that the lowest mean value of MIP-1α was found in the control healthy periodontium group and it increased with stages of periodontitis' progression with significant difference among groups. These results agree with Al-Sabbagh, et al. [18] and Sexton, et al. [19], where they found that saliva from periodontitis patients contained higher concentrations of MIP-1α than healthy controls.

Macrophage inflammatory protein-1α works by recruitment of inflammatory cells to inflamed sites, increasing the expression of MIP-1α by cells of the gingival epithelium to encourage an acute inflammatory response, and recruitment of B lymphocytes in later stages of periodontal disease which indicate that MIP-1α plays a vital part in leukocytes recruitment at initial and advanced periodontitis stages [20]. MIP-1α can furthermore stimulate osteoclastogenesis and induce formation of osteoclasts. MIP-1α stimulates bone resorption mainly through its CCR1 receptor, which is principally expressed by osteoclasts [21].

Regarding Pearson's correlation coefficient between MIP-1α and clinical periodontal parameters in study groups, there was a significant moderate positive correlation of MIP-1α with PLI in stage III periodontitis group. This finding agrees with [22], where their study revealed significant positive correlation between salivary MIP-1α and PLI, and disagrees with where no correlation existed between salivary MIP-1α and PLI. The reason for this correlation may be because of the elicited inflammatory host response in the presence of microbial plaque which involves an influx of PMN cells and macrophages. The exposure to bacterial LPS causes inflammatory cells to initiate the stimulation of inflammatory cytokines and chemokines expression in gingival connective tissues. Experiments have shown that MIP-1a expression is induced by PMN cells and gingival epithelial cells when subjected to bacterial LPS and IL-1β. MIP-1α is PMNs leukocytes chemotactic in acute inflammation and it is monocytes stimulatory in relation to osteoclastogenesis. MIP-1α's action in osteoclasts activation can moreover be synergized by IL-1β.

CONCLUSION

Salivary MIP-1α level is significantly higher in periodontitis groups than healthy group and their levels increase with increased severity of periodontitis which means MIP-1α can be used to be used to differentiate different stages of periodontitis.

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