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Estimation of Salivary MMP-8, MMP-9, MMP-13 and TIMP-1 in Chronic Periodontitis in Mosul

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Abstract The aim of the study was to estimate salivary concentrations of MMP-8, MMP-9, MMP-13, and TIMP-1in chronic periodontitis and control patients, and correlate periodontal parameters with salivary biomarkers in chronic periodontitis patients. The study group consisted of 44 patients suffering from chronic periodontitis, aging between ≤ 16-45 years old and 40 control samples collected from healthy individuals ranged between 16-40 years old. Chronic periodontitis was assessed on the basis of several periodontal parameters, including probing pocket depth (PPD), clinical attachment loss (CAL), bleeding on probing (BOP) and plaque index (PI). 5ml of unstimulated saliva was collected from patients and control groups to measure salivary biomarkers by ELISA technique. All periodontal clinical parameters were significantly higher in chronic periodontitis patients compared to control group ($p \leq 0.000$). Salivary MMP-8, MMP-9 and MMP-13 concentrations showed significant increase in chronic periodontitis ($p \leq 0.000$), while salivary TIMP-1 level showed significant increase in control group comparing with periodontitis ($p \leq 0.000$). Salivary MMP-13and MMP-9 showed significant difference in concentrations with different PPD, and CAL levels, while MMP-8 showed no significant difference, whereas all salivary biomarkers showed no significant difference with BOP index. According to Plaque index, MMP-8 concentration showed highly significant difference with different PI values.

Keywords: *Chronic periodontitis, MMPs, TIMP-1, periodontal parameters*

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1. Introduction

Chronic periodontitis is most common form of periodontitis, commonly seen in adults but can occur in children and adolescent, and characterized by slow to moderate rate of progression, gingival swelling, gingival inflammation, uneven destruction of alveolar bone and some recession [1]. Although microorganisms (mainly anaerobic bacteria) are considered as instigating agents, the disease progression is influenced by the host response together with environmental and behavioral factors [2]. Chronic periodontitis was further classified as localized disease where in ≤ 30% of evaluated sites demonstrate attachment and bone loss, or as a generalized disease where in >30% of sites are affected [3]. The disease may also be classified according to the severity of the disease as: slight when there is 1-2 mm of CAL, moderate 3-4mm of CAL or sever 5-6mm of CAL [4]. Saliva is an important biological fluid that contains both local and systemically derived biochemical substances used for detecting periodontal disorder the determination of biomarkers in saliva becoming an indispensable part of laboratory diagnostics and in the prediction of periodontal and other diseases [5]. Human MMPs are proteolytic enzymes responsible for the degradation of most ECM proteins during physiological organogenesis, growth, apoptosis, bone remodeling and wound repair as well as pathological processes like inflammatory diseases, including periodontal disease, caries, and rheumatoid arthritis [6]. Additionally they influence cellular proliferation, adhesion, migration [7], growth factor bioavailability, chemotaxis and signaling; and they are crucial for angiogenesis, vasodilation, tumorigenesis [8], metastasis [9], immunity, inflammation, and wound healing [10]. In humans, 23 different MMPs have been identified [11]. MMPs are primarily inhibited by their endogenous inhibitor, the tissue inhibitor of metalloproteinase (TIMP) [12], which are natural tissue inhibitors of MMPs and are divided into four types (TIMP-1, -2, -3, and -4). TIMPs inhibit MMPs by affecting catalytic zinc via the *N*-terminal cysteine residue and protein-protein surface interactions, TIMP are found in most tissues and body fluids and their expression is regulated during development and tissue remodeling [13]. Any imbalance between tissue inhibitors and MMPs initiates the destruction of collagens present in the gums, leading to chronic periodontitis [14].

2. Materials and Methods

2.1 Subject Groups

The subjects consisted of 44 patients with chronic periodontitis aging between 16 - 45 years old, attended to Periodontal Clinic in the College of Dentistry/Mosul University from January 2019 to May 2019. Women, smokers, and individuals with either acute or chronic medical illness or on oral medication for the last three months were excluded, while control group consisted of 40 clinically healthy individuals ranged between 16-40 years old. This group had no signs of any systemic disease, with clinically healthy periodontium . All patients with the control group underwent an oral examination.

2.2. Data Source

Case sheet was performed especially for the purpose of examination.

2.3. Saliva Collection

Unstimulated saliva was collected from patients and control groups via passive drooling into a sterilized disposable collector cup. Saliva was centrifuged at 3000 rpm for 20min. The clear supernatant fraction was then separated and dispensed in Eppendorff tubes and stored at -70˚C until required for analysis [15]. The parameters tested included probing pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP), and plaque index (PI). PPD represent the distance from the gingival margin to bottom of the pocket. The distance was calculated with a WHO probe, the pocket depth was measured at four sites of each tooth. CAL represent the distance between the bases of the pocket to the cemento-enamel junction, while BOP was calculated using the formula score 0= no bleeding after running periodontal probe, score 1= immediate bleeding or bleeding within 10 seconds of running periodontal probe.

pI was determined using different scores; score 0= no plaque on the tooth surface, score 1= film layer of plaque, no visualization, only by probe, score 2= plaque seen by visual inspection and by running probe, and score 3= abundance amount of plaque exceed cervical third of crown.

2.4. Materials and Kit Used for Salivary Serological Study

1. Human MMP8 ELISA test kit, Abcam. UK.
2. Human MMP9ELISA test kit, R&D System. USA.
3. Human MMP13 ELISA test kit, Abcam. UK.
4. Human TIMP-1ELISA test kit, R&D System. USA.

2.5. Statistical Analysis

T-Test, Anova test, correlations, Mean, Std Deviation, and Std Error.

3. Results

3.1. Comparison between Study and Control Groups in Relation to Periodontal Indices

The comparison between study and control groups using periodontal parameters (PPD, BOP, CAL, and PI), showed increasing in periodontal parameters in the study group comparing to control group, and the difference was highly significant ($P \leq 0.001$) (Table 1).

3.2. Comparison between Study and Control Groups in Relation to Salivary Parameters

The comparison between study and control groups using salivary parameters (MMP-8, MMP-9,and MMP-13), showed increasing in salivary parameters in the study group compared to control group, and the difference was highly significant ($P \leq 0.001$), while TIMP-1 showed increase in control group compared to control group and the difference was highly significant ($P \leq 0.001$) (Table 2).

Table 1. Student's t–test Comparison between Study and Control Groups

Parameters	Group	No.	Mean	± SD	t–value	Df	*p*–value
Plaque Index	Study	44	1.4205	0.50050	6.114	82	0.000*
	Control	40	0.6377	0.37616			
Bleeding on Probing	Study	44	0.7227	0.18535	26.759	82	0.000*
	Control	40	0.1465	0.12899			
Periodontal Pocket Depth	Study	44	4.6591	0.56828	17.547	82	0.000*
	Control	40	1.4275	1.51995			
Clinical attachment loss	Study	44	2.6591	0.50864	3.384	82	0.000*
	Control	40	0.4775	0.56828			

* Significant difference existed at $p \leq 0.05$.

Table 2. Student's t–test Comparison between Study and Control Groups

Salivary Parameters	Group	No.	Mean	± SD	t–value	*p*–value
MMP-8 pg/ml	Study	44	2.03082	0.457576	-12.794	0.000*
	Control	40	0.54282	0.296969		
MMP-9 Pg/ml	Study	44	0.86453	0.490186	-5.266	0.000*
	Control	40	0.19707	0.26935		
MMP-13 Pg/ml	Study	44	25.19512	4.997329	-12.533	0.000*
	Control	40	12.03720	1.606729		
TIMP-1 Pg/ml	Study	44	0.35052	0.163369	7.612	0.000*
	Control	40	2.00260	1.072811		

* Significant difference existed at $p \leq 0.05$.

3.3. Relationships between Salivary Parameter Concentrations and Periodontal Pocket Depth in the Study Group

The comparison between salivary parameters (MMP-8, MMP-9, MMP-13) levels and their relation with periodontal pocket depth within study group, showed significant difference in MMP-13, MMP-9 concentrations in patients with different PPD levels, while there was no significant difference in MMP-8 in patients with different PPD levels (Figure 1).

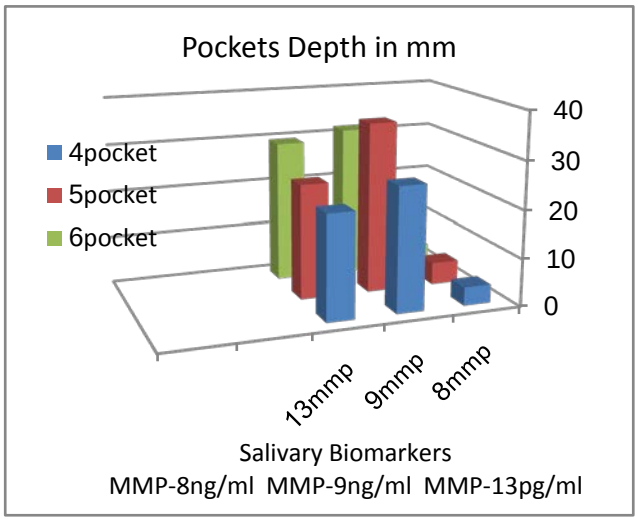

Figure 1. Relationships between salivary parameters concentrations and periodontal pocket depth in the study group

3.4. Relationships between Salivary Parameter Concentrations and Bleeding on Probing Index in the Study Group

The comparison between salivary parameter (MMP-8, MMP-9, MMP-13) levels and their relation with BOP within study group, showed no significant difference in MMP-8, MMP-9, and MMP-13 concentrations in patients with different BOP level (Figure 2).

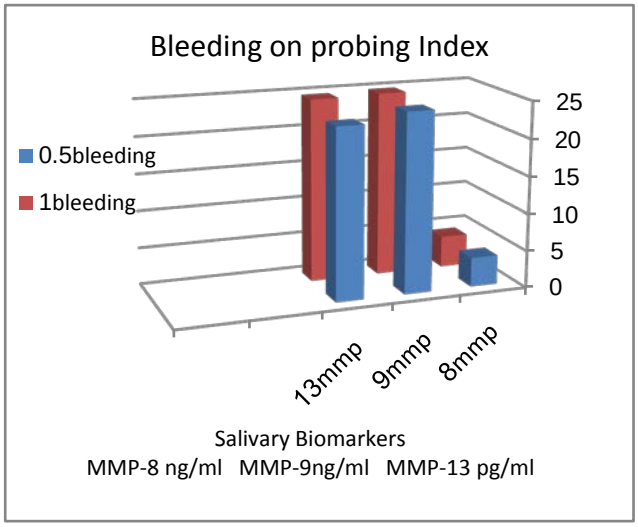

Figure 2. Relationships between salivary parameter concentrations and bleeding on probing in the study group

3.5. Relationships between Salivary Parameters Concentrations and Clinical Attachment Level in the Study Group

The comparison between salivary parameter (MMP-8, MMP-9, MMP-13) levels and their relation with CAL within study group, showed significant difference in MMP-13and MMP-9 concentrations in patients with different CAL levels, while there was no significant difference in MMP-8 concentrations patients with different CAL levels (Figures 3).

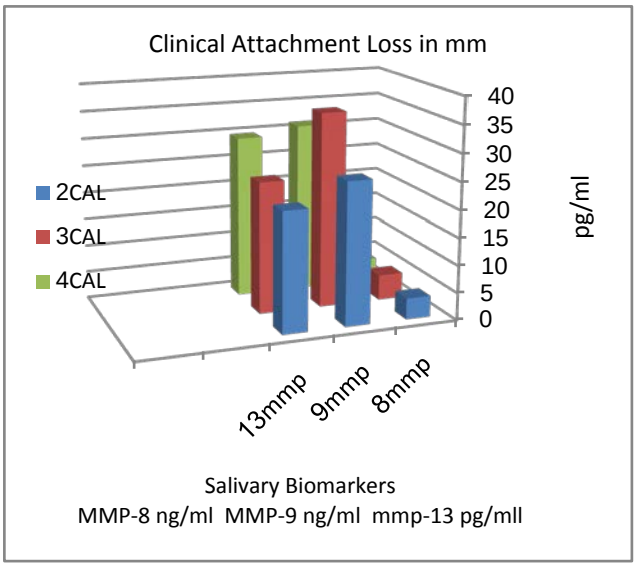

Figure 3. Relationships between salivary parameters concentrations and clinical attachment level in the study group

3.6. Relationships between Salivary Parameter Concentrations and Plaque Index in the Study Group

The comparison between salivary parameter (MMP-8, MMP-9, MMP-13) concentrations and their relation with PI within study group, showed significant difference in MMP-8 in patients with different PI values, while there was no significant difference in MMP-13 and MMP-9 in patients with different PI values (Figure 4).

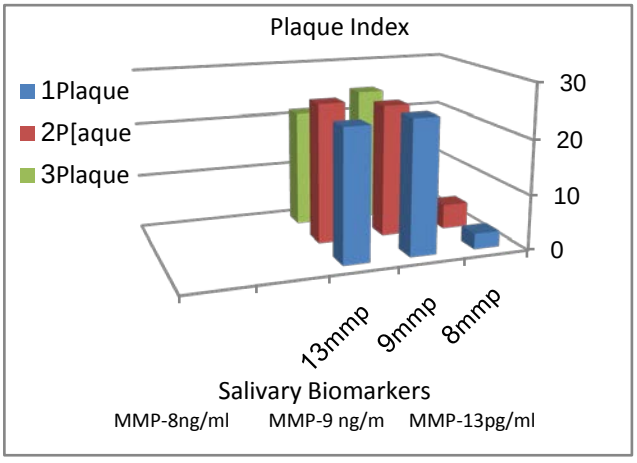

Figure 4. Relationships between salivary parameters concentrations and PI value in the study group

4. Discussion

The present study showed elevation of mean values of all clinical periodontal parameters (PI, CAL, BOP, and PPD) which were highly significant difference ($P \leq 0.001$) among the study group in comparison with control group. The similar finding was obtained by others [16].

The increase mean value of PI explained the role of the pathogen in the development and severity of PD. This is clear since plaque is the essential etiological factor in PD and is supported by the fact that the microbial biofilm is considered the primary and the major etiological factor responsible for initiation of periodontal disease [17]. The highest PI mean was found in study group. This could be related to the abnormally shaped gingival recession and the periodontal pocket formation in this group which may increase the plaque accumulation.

The result of BOP index, indicated the effect of plaque accumulation on blood circulation and the actual pathophysiological process that happened more in inflamed periodontal tissue compared to the clinically healthy periodontal tissue. In addition, the severity of bleeding and the influence of its incitement depends on the intensity of the inflammation where more accumulation of plaque with increased number of active sites that occur with chronic periodontitis [18].

The results of PPD and CAL in the present study, were expected in chronic periodontitis group which could be due to increase in the bacterial invasion and the amount of plaque that triggered destruction of the sulcular, junctional epithelium and collagen fibres resulted in apical migration of the clinical attachment level and increasing of probing depth [19]. The results of the present study illustrated increased salivary concentrations of MMP-8, MMP-9, and MMP-13 in patients with chronic periodontitis compared to control group, was statistically highly significant difference (P< 0.001). These results are similar to that reported by others [20]. A primary detachment of interstitial collagens by collagenases (MMP-8 and MMP-13) is considered to serve as a key step in the periodontal lesion amelioration [21], and cleavage of collagen I by MMP-13 seems to be the initial step of the entire bone resorption process [22]. Subsequent, denature collagen fragment degraded by gelatinase MMP-9. The higher concentration of TIMP-1 in control group reflecting the capability of these individuals to counter the activity of MMPs as evidenced by healthy periodontium with normal clinical parameters, while in patients with periodontal disease, it was inferred that a great amount of MMP was reflected through decrease TIMP activity, also evidence by the destruction of periodontal tissues and altered clinical parameters. A higher concentration of MMP-8 was found in patients with PI level 2, may be explained by the plaque accumulation which leads to gingival inflammation releasing of different pro-inflammatory mediators, and destruction of periodontal tissues and alveolar bone.

A higher concentration of MMP-9 and MMP-13 was found in patients with higher level of PPD and CAL which resulted from destruction of collagen fibres that leads to apical migration of the clinical attachment level and an increase of probing depth.

5. Conclusion

Salivary biomarkers (MMP-8, MMP-9, MMP-13, andTIMP-1) can help in early diagnosis or detection of chronic periodontitis, so this can resist the disease progression. This can positively affect the health of the individual as untreated chronic periodontitis causes pain, swelling, bleeding of gingiva, loosing of teeth and tooth loss.

References

[1] Mlachkova, AM., and Popova, CL. Efficiency of nonsurgical periodontal therapy in moderate chronic periodontal. *J Folia Med*. 56(2): 109-115. 2014.

[2] Lepilahti, JM., Ahonen, M-M., Hernandez, M., Munjal, S., Netuschil, L., and Uitto, VJ. Oral rinse MMP-8 point of care immune test identifies patents with strong periodontal inflammatory burden. *Oral Dis*. 17: 115-122. 2011.

[3] Armitage, G. C., and Cullinan, MP. Comparison of the clinical features of chronic and aggressive periodontitis. *Periodontol 2000*. 53: 12-27. 2010.

[4] Newman, GM., Takei, HN., Carranza, FA., and EDS. Carranza Clinical Periodontology. Philidalphia. 12th ed. pp 96-112. 2015.

[5] Janet. Oral fluid-based biomarkers of alveolar bone loss in periodontitis. Ann New York Acad. Sci. 1098: 230-251. 2007.

[6] Amalinei, C., Caruntu, I. D., Giusca, S. E., and Balan, R. A. Matrix metalloproteinases involvement in pathological conditions. *Rom J Morphol Embryol*. 51,215-228. 2010.

[7] Luo, K.w.; Wei, C., Lung, W.Y., Wei, X.Y., Cheng, B.H., Cai, Z.M., and Huang, W.R. EGCG inhibited bladder cancer SW780 cell proliferation and migration both in vitro and in vivi via down-regulation of NK-ᵏB and MMP-9. *J Nutr Biochem*. 41: 56-64. 2016.

[8] Stawoczyk, K. M., Weiienestein, M.D., Lee, S.B., Yomtoubian, S., Durrans, A., Choi, H., and Huang, W.RMatrix metalloproteinase 14 promotes lung cancer by cleavage of Heparin-Binding EGF-like Growth Factor. *Neoplasia*. 19: 55-64. 2016.

[9] Shintani, T., Kusuhara, Y., Daizumoto, K., Dondoo, T.O., Yamamoto, H., Mori, H., Fukawa, T., Nakatsuuji, H., Fukumori, T., and Takahashi, M. The Involvement of Hepatocyte Growth Factor-MET-Matrix Metalloproteinase 1 Signaling in Bladder Cancer Invasiveness and Proliferation. Effect of the MET Inhibitor, Cabozantinib (XL184), on Bladder Cancer Cells. Urology. 2016.

[10] Butler, G. S., and Overall, C. M. Update biological roles for matrix metalloproteinase and new "intracellular" substrate revealed by degradomics. *Biochemistry*. 48: 10830-10845. 2009.

[11] Jackson, B. C., Nebert, D. W., and Vasiliou, V. Update of human and mouse matrix metalloproteinase families. *Human Genomics*. 4(3): 194-201. 2010.

[12] Brew, K., and Nagase, H. The tissue inhibitors of metalloproteinases (TIMP): an ancient family with structural and functional diversity. *Biochimica Biophysica Acta*. 1803(1): 55-71. 2010.

[13] Brew, k., Dinakarpandian, D., and Nagase, H. Tissue inhibitors of metalloproteinases: evolution, structure and function. *Biochimica Biophysica Acta*. 1477(1-2), 267-283. 2000.

[14] Sapna, G., Gokul, S., and Bagri-Manjrekar, K. Matrix metalloproteinases and periodontal diseases. *Oral Dis*. 20(6): 538-550. 2014.

[15] Aurer A, Jorgić-Srdjak K, Plančak D, Stavljenić-Rukavina A, and Aurer-Koželj J. Pro inflammatory factors in saliva as possible markers for periodontal disease. *Coll Antropol*. 29(2) 435-439. 2005.

[16] Khongkhunthian, S; Kongtawelert, P; Ongchai, S; Pothacharoen, P; Sastraruji, T; Jotikasthria, D and Krisanaprakornkit, S. Comparison between Two Biochemical Markers in Evaluating Periodontal Disease Severity: A Cross-Sectional Study. *J of BMC Oral Health*. 14-107. 2014

[17] Lindhe, J; Karring, T and Niklans, P.L. Clinical Periodontology and Implant Dentistry. 5thEdition. Blackwell Munksgaard, a Blackwell Puplishing Company. Oxford. 2008.

[18] Carranza, F.A. Clinical Periodontology. 10th Edition. WB Sounder Company; Philadelphia. 2009.

[19] Newman, GM., Takei, HN., Carranza, FA., and EDS. Carranza Clinical Periodontology. Philidalphia. 12th ed. pp 96-112. 2015.

[20] Zhang, L., Li, X., Yan, H., and Huang, L. Salivary matrix metalloproteinase (MMP)-8 as a biomarker for periodontitis: A PRISMA-compliant systemic review and meta-analysis. *Medicine*. 97(3). 2018.

[21] Hernandez, M., Martinez, B., Tejerina, JM., Valenzuela, MA., and Gamonal, J. MMP-13 and TIMP-1 determinations in progressive chronic periodontitis. *J Clin Periodontol*. 34:729-735. 2007.

[22] Uchida, M., Shima, M., Shimoaka, T., and Fujieda, A. Regulation of matrix metallproteinases (MMPS) and tissue inhibitors of metalloproteinases (TIMPs) by bone resorptive factors in osteoblastic cells. *J Cell Physiol*. 185:207-214. 2000.