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Relation between salivary Matrix-metalloproteinase-8 with periodontal health; dental biofilm induced gingivitis, localized and generalized periodontitis

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Abstract

Aim of the study: was to find the potential relation between the salivary concentration of matrix metalloproteinase-8 with periodontal health and disease by examining its' level in individuals with healthy periodontium, with dental biofilm induced gingivitis and with periodontitis (localized and generalized).

Material and method: Saliva was collected from 90 participants with an age range from (20-60) years, all of them were systemically healthy. 25 cases were enrolled under the dental Biofilm induced gingivitis group, 25 cases under the localized periodontitis group, and 25 cases under the generalized periodontitis group, with 15 subjects enrolled in the healthy periodontium(control) group. Detection of MMP-8 in saliva was achieved by utilizing Enzyme-Linked Immunosorbent Assay (ELISA).

Results: The present data showed that MMP-8 was higher significantly($P<0.05$) in disease groups (dental Biofilm induced gingivitis, localized and generalized periodontitis groups) as compared to the healthy (control) group. With a significant difference between dental biofilm-induced gingivitis and generalized periodontitis groups.

Conclusion: There is a positive relationship between salivary matrix metalloproteinase-8 with periodontal health and disease.

Keywords: Gingivitis, Matrix metallo-proteinase-8, Periodontitis, Saliva.

Introduction:

Bacteria, environment and the host defense mechanism exclusively are the main factors that lead to periodontal diseases which are one of the most popular chronic inflammatory disorders that affect humanity, nonetheless, the host response caused enough soft and hard tissue damage. This might lead to tooth loss in severe conditions [1].

The periodontal tissue is affected by two categories of periodontal diseases:

1. Dental Biofilm induced gingivitis: such inflammation remains confined to the gingiva and does not approach the periodontal attachment (cementum, bone, and periodontal ligament) beyond the mucogingival junction and is opposed by the reduction of plaque biofilm at and apical to the gingival margin. Usually result from the interplay between plaque biofilm and inflammatory host defense [2].

2. Periodontitis: represented by the extension of the gingival inflammation toward the periodontal attachment causing clinical attachment loss as a result of microbial, host defense-mediated inflammation [3]. According to the extent, periodontitis can be categorized [4]:

   ➤ "Molar/incisor pattern" if the bone loss limited to the molar/incisor.
   ➤ "Localized periodontitis" is where the bone loss caused by periodontitis effect ≤ 30% of the teeth.
   ➤ "Generalized periodontitis" is where the bone loss caused by periodontitis effect > 30% of the teeth.

In the attempt to move the periodontal examination from traditional methods to biomarkers' recruitment for the qualification and quantification of the clinical information objectively [5]. A biomarker was defined by the National Institutes of Health Biomarkers Definitions Working Group as a characteristic that is calculated and estimated objectively to refer to natural biological and pathological processes or pharmacological feedback to a curative medication [6]. Biomarkers were useful for understanding human or animal diseases; and for their prevention, diagnosis, treatment, and, prognosis [7].

MMPs are proteolytic enzymes that belong to the zinc protease superfamily, physiologic remodeling of matrix proteins and basement membranes are roles of them [8]. MMP-8 (matrix-metalloproteinase-8/collagenase-2) is part of this family as a member of the collagenase group with an exclusive ability to break down type I and III collagen [9]. Mainly, de-granulated poly-morphonuclear leukocyte liberates up to 20% of its' content as MMP-8 [10]. Other non-PMN lineage sources of MMP-8 are fibroblasts, macrophages, endothelial cells, smooth muscle cells, and epithelial cells [11], [10], [12].

There are two varieties of MMP-8: latent and active form, the first one is mainly related to health, and the other upturns in reply to periodontal/peri-implant [11], [13]. Certain pathogens (Treponema denticola; and Tannerella forsythia) are included in the induction of the inflammatory cascade related to MMP-8 production [14].

A research done by (Syndergaard et al., 2014) [15] showed a minor up-regulation of MMP-8 in the gingivitis group. After proper dental prophylaxis, MMP-8 is down-regulated. MMP-8 is a member of the main collagenolytic enzymes related to the devastation of periodontal/peri-implant tissue and the advancement of disease [16].

Saliva is a hypotonic bio-fluid, a mixture of salivary glands, gingival crevicular fluid and exudate from oral mucosa [17]. It has an essential role in maintaining a healthy oral cavity [18]. Saliva is a beneficial fluid for diagnosis in vitro and also clinically, for

monitoring prognosis, patients with systemic or oral diseases to show the response to treatment as it contains specific biomarkers [19]. The collection of salivary fluid is quick, effortless, inexpensive, and non-invasive [20].

The study aimed to find the potential relation between the salivary concentration of matrix metalloproteinase-8 with periodontal health and disease by examining its' level in individuals with healthy periodontium, with dental biofilm induced-gingivitis and with periodontitis (localized and generalized).

3. Material and method
Study design and ethics

The study design was case-control that took place from January 2022 to June 2022 at the Teaching Clinics of the Periodontics Department, College of Dentistry, University of Baghdad. At the University of Baghdad, College of Dentistry, Ethics Committee reviewed the study and granted it ethical approval (Study No.457622). For the goal of obtaining salivary samples, 90 participants (males and females) were examined and separated into the following four groups:

- Group A: Healthy periodontium as a control group included 15 subjects who had BOP (bleeding on probing) < 10%, PPD (probing pocket depth) ≤3 mm on intact periodontium (no clinical attachment loss) [2].
- Group B: Dental Biofilm induced gingivitis included 25 patients who had generalized gingivitis BOP >30%, PPD ≤3 on intact periodontium (no clinical attachment loss) [2].
- Group C: Localized periodontitis where the bone loss caused by periodontitis affects ≤30% of the teeth, including 25 patients of unstable periodontitis with PPD ≥5mm or PPD ≥4mm and BOP of the pocket [4].
- Group D: Generalized periodontitis where the bone loss caused by periodontitis effect > 30% of the teeth,

including 25 patients of unstable periodontitis with PPD ≥5mm or PPD ≥4mm and BOP of the pocket [4].

All cases of periodontitis were characterized by interdentally detectable CAL (clinical attachment loss) at ≥2 non-adjacent teeth or CAL present at ≥3mm on the buccal (facial) or lingual/palatal surfaces in conjunction with pocketing >3mm at ≥2 teeth [3].

Salivary samples were used to compare the salivary levels of MMP-8 in cases with dental Biofilm induced gingivitis and periodontitis(localized and generalized) to healthy controls. Assessing clinical parameters and relating them to the concentration of the chosen biomarker.

Inclusion and exclusion criteria

In our study, we needed systemically healthy patients with at least 20 teeth who were not taking medications for the previous 3 months. For the exclusion criteria: individuals with systemic diseases, individuals with any previous extensive periodontal therapy or presently under active periodontal therapy, individuals on an antibiotic or immunosuppressant medications within the previous 3 months, individuals presented necrotizing ulcerative gingivitis, aphthous ulcer or any lesion not related to the disease, individuals who were smokers or alcoholics, individuals with an orthodontics or dental implant, pregnant or lactating mothers, women on contraceptive pills.

2.3 Periodontal Parameters and clinical examination

Inter- and intra-examiner calibration for categorical variables (PI and BOP) were assessed by using a kappa-coefficient assay. The targeted level was kappa value ≥75% to decide a good level of agreement was present. For continuous variables (PPD and CAL), the level of agreement rounded to the nearest millimeter should be >0.9 as determined by Interclass coefficient assay.

Except for the wisdom teeth, all other teeth were subjected to periodontal examination. This included Full mouth plaque score(FMPS) [21], Bleeding on Probing(BOP) [22], Probing pocket depth(PPD) [23], Clinical Attachment Level (CAL) [24] utilizing a periodontal probe (Michigan O probe), marking at 1, 2, 3, 5, 7, 8, 9, and 10 mm at six places per tooth except for a full mouth score (FMPS) which covered four surfaces of the tooth. FMPS was applied with the aid of disclosing agent to determine the presence/absence of plaque where a score of 0 was given to the surface that didn't stain and a score of 1 was given to the surface stained purple due to plaque accumulation. This parameter was used for ease of application and time-saving.

The staging of periodontitis was assessed by employing the tooth with the worst clinical attachment loss. The extent was determined by partitioning the number of teeth with clinical attachment loss by the total number of teeth [3].

Salivary sample collection
Unstimulated saliva was gathered into a sterile test tube from all participants. The volume of saliva eliminate any cellular debris from the salivary samples. Following the manufacturer's instructions and using (Human Reader HS, HUMAN Society for Biochemical and Diagnostica mbH, Wiesbaden, Germany), saliva samples then will be examined for the protein level of MMP-8 by using commercially available ELISA kits that were ordered from BioSource in California, USA. The concentrations were then stored and transferred to spreadsheets for analysis.

Statistical analysis
For the continuous data, descriptive statistics including mean, and SD (standard deviation), were applied, while for categorical variables, frequency and percentage were applied. The distribution of the data was investigated by the Shapiro-Wilk test. Since all the data regarding clinical periodontal parameters entered were not

normally distributed, to compare data of clinical periodontal parameters (PI, BOP) among groups, the Kruskal-Wallis test was used and in case of significance, multiple pairwise comparisons were done and for data comparison of PPD and CAL between periodontitis groups, Mann Whitney U test was applied. The data of MMP-8 was not normally distributed, Therefore, the Kruskal-Wallis test was used for the comparison of MMP-8 level among groups and in case of significant results, multiple pairwise comparisons were carried out. To correlate the biomarker with periodontal parameters, the Spearman correlation coefficient was applied since all of the data inserted was not normally distributed. The level of significance was set at $P< 0.05$. The analysis was done by using SPSS software (version.25).

Results
The age of the participants ranges between (20-60) years with a mean (33.733±11.987). Gender distribution throughout the sample showed that the female had a higher percentage. Comparing data of (PI) among groups has shown a significant difference between them (P=0.000). Multiple pairwise comparisons among patients showed higher values of PI among disease groups when compared to the healthy (control) group also there was an insignificant difference between the disease groups. For BOP, the same findings as PI except for the comparison between the disease groups showed a significant difference between groups C and D. For PPD and CAL, their data comparison between periodontitis groups showed a significant difference between them with group D having the highest value as compared to group C. Descriptive statistics of age, gender, and clinical periodontal parameters among groups are shown in Table-1, and multiple pairwise comparisons of PI, and BOP among groups are shown in Table-2, and Table-3 respectively. Mann Whitney U test for data comparison of PPD, and CAL are shown in Table-4, and Table-5 respectively.

Data comparison of MMP-8 levels among groups showed a significant increase in MMP-8 levels among disease groups in comparison to the healthy (control) group. Also, there was a significant difference between group B and group D. (Figure-1)

When we correlated salivary MMP-8 concentration with clinical periodontal parameters, results presented that MMP-8 was significantly and positively correlated with all periodontal parameters with correlation coefficients (r) for the four parameters (PI, BOP, PPD, CAL) 0.253, 0.370, 0.440, 0.490 respectively. Table-6.

Discussion

According to the data collected, it was revealed that with increasing age, the severity of periodontal diseases increased. The human cumulative oral history is determined by the duration of time in which the periodontium is exposed to bacterial plaque which in turn explains why the severity of periodontal disease and alveolar bone injury increased with age [26].

Gender distribution throughout the sample showed that the female has a higher percentage. These findings agree with these researches (Mohammed et al., 2022) [27], (Aldhaher et al., 2018) [28]. Alteration of bacterial flora introduced by female sex hormones makes them more prone to gingivitis and periodontal diseases [29].

Data comparison of PI among groups findings coincide with these studies (Al-Karawi and Al-Rubaie, 2014), (Salman, 2015), (Mousa and Saliem, 2016) [30], [31], [32]. This could be because neglecting oral hygiene has led to the apposition of food deposits (plaque Biofilm) that causes the inflammation of the supporting tissue of the teeth. Dental plaque is considered the main cause of periodontal disease [33].

For the BOP index, data comparison findings coincide with these studies (Rai et al., 2008), (Rangbulla et al., 2017) [34], [35]. The severity of bleeding relies on how would plaque aggregation influences blood circulation, pathophysiological changes that are more pronounced in inflamed periodontal tissue compared to clinically healthy periodontal tissue also relies on the intensity of this inflammation [36].

For PPD and CAL, data comparison findings coincide with these studies (Gupta et al., 2015), (Salman, 2015) [37], [31]. Sulcular and junctional epithelium extermination and the following bone loss in periodontitis might be due to the amount of plaque and accompanied by increased bacterial invasion [38].

Data comparison of MMP-8 among groups findings coincide with the following researches (Gupta et al., 2015), (Rangbulla et al., 2017) [37], [35]. The reason behind increasing the level of MMP-8 in periodontitis that is MMP-8 has a unique ability to destruct types I and III collagen, which is a critical action during periodontal devastation in periodontitis but not for normal tissue remodelling. It is noteworthy to mention that MMP-8 is a superior marker of inflammation and it is mainly released from neutrophils in the oral fluids [37]. One study done by (Romero-Castro et al., 2020) [39] showed decreasing the level of MMP-8 level among the disease groups in comparison to the healthy (control) group because as part of tissue remodelling in a healthy state, MMP-8 is released as a total form while in the disease state active form dominates.

MMP-8 correlated significantly and positively with all clinical periodontal parameters. This finding agrees with these studies (Rai et al., 2008), (Gupta et al., 2015) [34], [37]. The inflammatory process excites an immunological response that leads at once to the production of MMP-8 which in turn causes collagen degradation [40]. Certain pathogens (Treponema denticola, and Tannerella forsythia) are included in the induction of the inflammatory cascade related to MMP-8 production [14]. MMP-8

(neutrophil-derived major interstitial collagenase) is one of the MMPs (MMP9, MMP-13, MMP8) that is involved in periodontal destruction affecting saliva, GCF, gingival tissue, and mouth rinse samples [41]. Also, Herr et al., 2007 [42] concluded that the host's cell-derived interstitial collagenases cleaved the gingival and PDL collagens during periodontal progressive devastation. Saliva had a highly upgraded level of MMP-8 in individuals with periodontal disease.

Limitations of the study were the small sample size and the use of saliva as a sample since it gives no information about the active site of the disease. Also, the application of an ELISA kit to measure the biomarker level since it is a sensitive technique and the procedure would be associated with false positive and false negative results. The exclusion of diabetic and smoker patients from the study as diabetes and smoking are important risk factors for periodontal diseases.

In Conclusion:

There is a positive relationship between salivary MMP-8 with periodontal health and disease.

Suggestions:

Include other biomarkers like MMP-13 that are included in the destructive process and correlate it with periodontal health and disease. Measuring the active form of the biomarker since it is more precise in predicting the activity/ severity of the disease. Applying another oral fluid as a sample like gingival crevicular fluid.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Table-1: Descriptive statistics of gender, age, and clinical periodontal parameters among groups.

Gender	Descriptive statistics	Group A n =15	Group B n =25	Group C n =25	Group D n =25	Total n =90
Male	Frequency (n)	2	15	7	12	36
	Percentage %	13.3 %	60 %	28 %	48 %	40 %
Female	Frequency (n)	13	10	18	13	54
	Percentage %	86.7 %	40 %	72 %	52 %	60 %
Age	Mean± SD	23.600± 2.746	24.440± 5.575	36.240± 8.847	46.600± 9.273	33.733± 11.987
Clinical periodontal parameters						
PI	Mean± SD	0.075± 0.017	0.844± 0.194	0.754± 0.125	0.792± 0.238	
	Median	0.076	0.92	0.76	0.85	
BOP	Mean± SD	0.05± 0.011	0.538± 0.112	0.444± 0.091	0.569± 0.172	
	Median	0.05	0.523	0.446	0.57	
PPD	Mean± SD	-----	-----	4.494± 0.480	5.350± 1.424	
	Median	-----	-----	4.4	4.840	
CAL	Mean± SD	-----	-----	2.362± 0.780	3.766± 0.815	
	Median	-----	-----	2.380	3.610	

-SD: standard deviation, Group A: healthy periodontium(control), Group B: dental Biofilm induced gingivitis, Group C: localized periodontitis, Group D: generalized periodontitis, PI: plaque index, BOP: bleeding on probing index, PPD: probing pocket depth, CAL: clinical attachment loss.

Table-2: Multiple pairwise comparisons of PI among groups

Groups	Test statistics	Significance	Adj. significance
Group A- Group B	-52.280	0.000	0.000
Group A- Group C	-35.860	0.000	0.000
Group A- Group D	-46.860	0.000	0.000
Group C- Group D	-11.000	0.136	0.814
Group C- Group B	16.420	0.026	0.155
Group D- Group B	5.420	0.462	1.000

*The significance level is 0.05, the significance value has been adjusted by the Bonferroni correction by multiple tests.

Table-3: Multiple pairwise comparisons of BOP among groups

Groups	Test statistics	Significance	Adj. significance
Group A- Group B	-48.860	0.000	0.000
Group A- Group C	-33.120	0.000	0.001
Group A- Group D	-53.020	0.000	0.000
Group C- Group D	-19.900	0.007	0.042
Group C- Group B	15.740	0.033	0.199
Group B- Group D	-4.160	0.573	1.000

*The significance level is 0.05, the significance value has been adjusted by the Bonferroni correction by multiple tests.

Table-4: Mann-Whitney U test for PPD data comparison between periodontitis groups.

Groups	Mean rank	Mann-Whitney U	Significance
Group C	20.84	196	0.023
Group D	30.16		

*Significance level at 0.05.

Table-5: Mann-Whitney U test for CAL data comparison between periodontitis groups.

Groups	Mean rank	Mann-Whitney U value	Significance
Group C	15.4	60	0.000
Group D	35.6		

*Significance level at 0.05.

Table-6: Correlation between salivary MMP-8 and clinical periodontal parameters.

		PI	BOP	PPD	CAL
MMP-8	significance	0.016	0.000	0.000	0.000
	Correlation coefficient	0.253*	0.370**	0.440**	0.490**

-Correlation is significant at the 0.05 level (2-tailed).*
 Correlation is significant at the 0.01 level (2-tailed).**
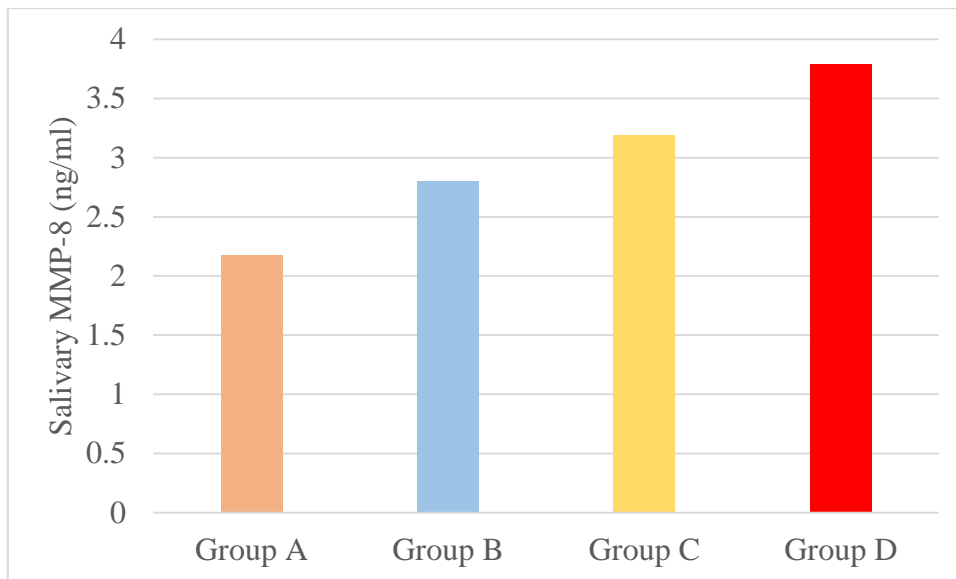-MMP-8: matrix metalloproteinase-8, PI: plaque index, BOP: bleeding on probing index, PPD: probing pocket depth, CAL: clinical attachment loss.

Figure -1: Salivary MMP-8 levels among groups.