Original Article

High Level of Matrix Metaloproteinase-9 and Toll-Like Receptor-4 in Gingival Cervicular Fluid of Periodontitis Patients

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Abstract

Background: Periodontitis is a prolonged inflammatory issue that destroys the connective tissue and bone that hold the teeth in place. This illness is regarded as one of the leading causes of tooth deterioration. Poor dental health can have a significant impact on overall health, pain, eating, chewing, smiling, and communicating. Furthermore, missing teeth have a substantial impact on people's quality of life and overall well-being. Severe periodontitis, which affects 5%–20% of the adult population worldwide, can result in tooth loss. **Objectives:** This study sought to determine the levels of matrix metaloproteinase-9 (MMP9) and toll-like receptor-4 (TLR4) in periodontitis patients' gingival cell fluid and how these levels connected to clinical attachment loss and probing pocket depth. **Materials and Methods:** Ninety samples were used in this study, including 60 samples; 38 of men and 22 of women who suffer from periodontitis, whereas 11 of men and 19 of women were considered as the control group. This study extended from December 2022 to February 2023. The age of patients and control were ranging from 30 to 63 years old. The samples were analyzed by the enzyme-linked immunosorbent assay testes technique to measuring level of MMP9 and TLR4. **Results:** The level of TLR4 and MMP9 was higher in patients than control concentrations (562.45 ± 70.06, 77.12 ± 11.51 pg/mL) and (8.07 ± 0.19, 6.36 ± 0.51 pg/mL), respectively. **Conclusion:** According to investigation, patients with periodontitis had a greater amount of MMP9 and TLR4 than healthy people.

Keywords: CAL, MMP9, periodontitis, PPD, TLR4

Introduction

Periodontitis is a multifactorial illness caused by bacteria that causes inflammation of tooth supporting tissue as well as loss of periodontal ligament attachment and bone support.[1]

Periodontitis is also defined a chronic multifactorial inflammatory disease that destroys both non-mineralized and mineralized connective tissues. Its etiology includes a complicated interplay between periodontal pathogens and host immunity, which is significantly impacted by genetic and environmental variables.[2]

Preventive regular teeth brushing and flossing at least twice daily consider the most effective ways to avoid oral disease and periodontitis by reduction of bacterial dental plaque.[3] Recently, it is shown that antioxidant diets such as vitamin C are a natural defense against many inflammatory diseases including periodontitis for preventing and delaying periodontal disease progression.[4]

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Periodontal bacteria cause periodontitis by proliferating in host cells and producing lipopolysaccharide (LPS), which is recognized by toll-like receptor-4 (TLR4) and encourages the immunological response to release pro-inflammatory, prostaglandin, proteinase, and matrix. Metalloproteinase (MMPs).[5] MMPs are multifunctional enzymes that participate in physiological processes such as embryogenesis, the regular remodeling of tissue, wound curing, and angiogenesis. Additionally, periodontitis, cancer, atherosclerosis, and rheumatoid arthritis are related to a pathological condition connected to MMP..[6] MMPs are also thought to have anti-inflammatory effects

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where the periodontitis was greatly exacerbated and the necrosis rate increased when MMPs expression was suppressed by chemical compounds. MMP9 contrarily starts osteoclast activation by removing collagen from the demineralized bone. As a result, its function is to moderate and encourage bone degradation.[7] According to the literature, it is appeared that the periodontitis has some important clinical periodontal markers, such as probing pocket depth (PPD) which is measured clinically as the distant between the gingival border and the pocket's base as well as clinical attachments loss (CAL), which is calculated clinically as the separation between the cement enamel junction and the pocket's base. CAL = probing depth + recession of the gingiva.[8] It can be concluded that the relationship between clinical periodontal parameters and their markers is incompletely understood. Thus, this study designed to determine the concentration of (MMP9 and TLR4) by enzyme-linked immunosorbent assay testes (ELISA) in gingival cell fluid (GCF) of periodontitis patients and correlate it with CAL and PPD.

Materials and Methods

Samples collection

Sixty samples (38 [63.33%] men and 22 [36.67%] women) were taken from patients with periodontitis who visited specialized dental centers at University of Babylon, College of Dentistry, Department of Periodontology and the control group consists of 19 (63.33%) women and 11 (36.67%) men. The age of those patients and control group is ranging between 30 and 63 [Figure 1].

Periodontal examination parameters

Bleeding on probing (BOP), plaque score (PS), PPD, and CAL were achieved by periodontologist of the clinic.

Gingival cervicular fluid collection

Swabs were collected from GCF for the immunological study by paper point and were placed in an Eppendorf tube containing 300 µL of phosphate buffer saline, centrifuged for 15 min, then the paper point was aloof from the tube by the sterile forceps, and the samples were stored at –20°C for immunological tests by the ELISA.[9]

Statistical analysis

After making the test of normality, the samples which underwent a normal distribution The statistical analysis was utilized. The SAS (2018) software (SAS Institute, USA) was used to determine the impact of various variables groups (patients and controls) on research parameters. The *t* test was employed to compare means substantially. Correlation coefficients were used to estimate between variables in this study.[10]

Ethical approval

The researched was conducted in accordance with the Helsinki Declaration's ethical principles. Before taking the sample, the patients analytical and verbal consent were obtained. To obtain this permission, a local ethics committee evaluated and approved the study protocol, subject information, and consent form using document number 6275 (containing the number and date in December 14, 2022).

Results

According to the results of Table 1, a significant difference was shown in comparison among all age groups of patients, whereas the patients groups 50–59 and 40–49 years old only recorded substantial changes in relation to control groups of that age.

According to the outcomes of Table 2, the statistical analysis generally demonstrated that all clinical parameters of periodontitis groups showed significantly increase compared to control.

Comparing the matrix metaloproteinase-9 patient and healthy groups

According to Figure 2, the results showed that a significant difference was found MMP9 level of patients compared to control group.

Table 1: The distribution of study samples according to age in patients and control groups

Age groups (year)	Patients (No = 60)	Control (No = 30)	*P* value
30–39	24 (40.00%)	28 (93.33%)	0.579 NS
40–49	15 (25.00%)	2 (6.67%)	0.0016 **
50–59	18 (30.00%)	0 (0.00%)	0.0001 **
≥60	3 (5.00%)	0 (0.00%)	0.384 NS
P value	0.0001 **	0.0001 **	–

NS: non-significant

** $P \leq 0.01$

Table 2: Clinical parameter of periodontitis patients and control group

Group	Mean ± SE			
	PD (mm)	CAL (mm)	PS	BOD (%)
Patients	3.46 ± 0.11	2.93 ± 0.13	35.65 ± 1.07	31.37 ± 1.19
Control	1.40 ± 0.08	0.00 ± 0.00	7.81 ± 0.38	5.60 ± 0.22
t test	0.329**	0.370**	3.082**	3.387**
P value	0.0001	0.0001	0.0001	0.0001

** $P \leq 0.01$

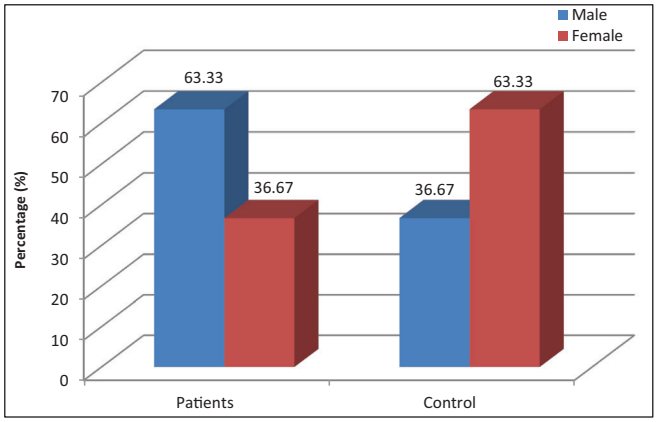

Figure 1: Distribution of study samples according to gender in patients and control

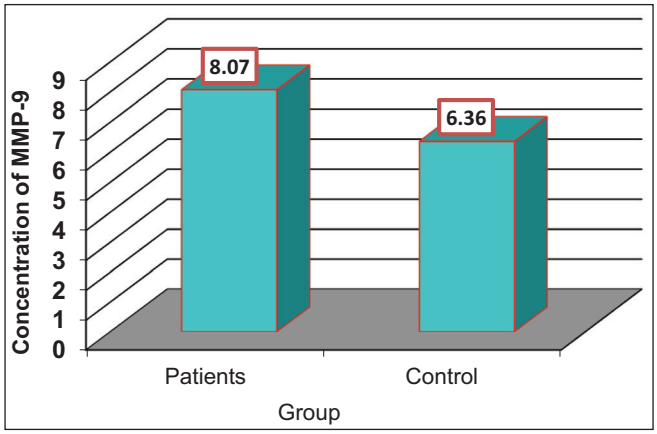

Figure 2: Comparison between patients and control groups in concentration of MMP9

Table 3: Correlation coefficient between PD, CAL with TLR4

Parameters	Correlation coefficient—*r*		
	PPD	CAL	*P* value
TLR4	0.12 NS	0.06 NS	0.754

NS: non-significant

* $P \leq 0.05$

Comparison among patients and healthy groups in toll-like receptor-4

According to Figure 3, the findings demonstrated that a significant difference was found TLR4 level of patients compared to their control.

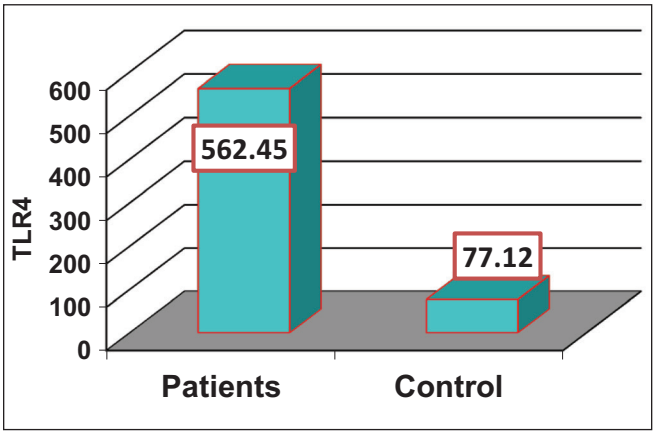

Figure 3: Comparison between patient and control in TLR4 levels

Table 4: Correlation coefficient between PD, CAL with MMP9

Parameters	Correlation coefficient—*r*	
	PPD	CAL
MMP9	–0.02 NS	–0.24 *

NS: non-significant

* $P \leq 0.05$

Correlation coefficient between probing pocket depth, clinical attachments loss with toll-like receptor-4

According to Table 3 about the correlation coefficient between PPD, CAL with TLR4, the outcomes of the statistical analysis demonstrated. There was no discernible change difference between CAL, PPD, and TLR4 level.

While the results of MMP9 in the Table 4 showed significant negative differences in comparison to CAL.

Discussion

Periodontitis is derived from the terms "periodont-," referring to "the structure that surrounds teeth," and "itis," which means "infection."[11]

The incidence and severity of periodontitis are both connected to aging, which has been explained by the fact that as people become older, their exposure to bacterial biofilm grows.[12]

Almost all organs undergo alterations as they age. Changes in the oral cavity with age are comparable to those seen elsewhere in the body, such as loss of muscle

tone and deterioration of both hard and soft tissues. Salivary composition varies with age, as demonstrated by decreasing salivary flow which contributes to periodontitis.[13]

As shown in Table 1, a significant difference was shown in comparison among all age groups of patients, whereas the patient groups 50–59 and 40–49 years old only recorded substantial variations in compared to the control groups of that age.

The current study is identical to previous studies[13-15] which found high age most effected with periodontitis.

Regarding to the gender of the participants of the current study as shown in Figure 1 a significant difference was found between infected males and females.

The current findings are consistent with previous results obtained by Chikte *et al*.[15] which found revealed men had poorer periodontal health than women. Similar findings were shared by Rajasekar *et al*.[16] Another study made by Khan[14] mentioned that men were more likely than women to have periodontitis, with rates of 27.6% and 18.1%, respectively, which may be duo to women consistently demonstrating better oral hygiene habits than men, brushing and flossing more often and smoking less. Poor oral hygiene practices such as insufficient tooth brushing and other oral hygiene habits can encourage bacterial deposition and dental plaque accumulation on teeth and gums, creating a favorable environment for inflammatory changes in periodontal tissues.[3]

The current investigation discovered a link between periodontitis and clinical periodontal characteristics (PPD, CAL, PS, and BOD) as mentioned in Table 2 that revealed significant differences in patients compared to the control in all periodontal parameters. The clinical parameter was measured according to Mazurek-Mochol *et al*.[17] Previous study achieved by Hamodat *et al*.[18] found that all periodontal parameters compared to controls, were considerably greater in patients. Another study made by Banu *et al*.[19] also approved with the current study and mentioned that periodontal indices including plaque index and pocket depth were discovered have considerably greater in periodontitis patients than in the healthy group which were compatible to other previous studies.[20,21]

Concerning to the level of MMP9, substantial MMP9 concentrations in patients differed from those in the control group as shown in Figure 2. It is revealed that one of the earliest steps of tooth-supporting tissue degradation is the breakdown of the extracellular matrix.[22] Because MMPs degrade all extracellular matrix proteins, specific knowledge of their existence, quantities, and activities is regarded as critical for a thorough understanding and characterization of various forms of periodontitis for determining active destruction and monitoring purposes.[23] This finding was compatible with a previous study that found an increase in MMP9 levels was reported in patients with progressive CAL.[24] Another research, which supported the present findings, found that patients had higher levels of MMP9 in their GCF than controls, and that these markers reduced following periodontal treatment.[23] Similar findings also obtained by Ghosh *et al*.[25] showed that increased concentration of MMP9 are closely linked to the severity and development of periodontal disease, particularly when they contribute to the alveolar bone resorption and periodontal tissue loss.

The current investigation discovered a substantial difference in the TLR4 levels of patients compared to their controls, as shown in Figure 3. The relationship between TLR4 expression and periodontitis severity is consistent with periodontal etiology. The immunological response to this receptor is stimulated by LPS from pathogenic bacteria that are multiplying in host cells. TLR4 then recognizes this receptor, which causes pro-inflammatory, prostaglandin, proteinase, and matrix MMPs to be produced.[5,22] Furthermore, the present results were consistent with another previous study which found that periodontitis had significant increases in TLR4 gene expression which lead to an elevation in the concentration of TLR4 in gingival tissues.[26] The difference was significant between the healthy and Periodontitis. Kikkert *et al*.[27] mentioned that periodontitis is a chronic infectious condition caused by gram-negative sub-gingival bacteria TLR4 receptors are stimulated by bacterial components and produce an immune response. However, the recent result differs from the study achieved by Dolly *et al*.[5] that mentioned that TLR4 should not be used as a clinical biomarker in periodontitis diagnostic tools since it was larger in shallow pockets compared to the control group but the different was not statistically significant.

As regards to the correlation coefficient between PPD and CAL with TLR4 of the current study [Table 3], there were no significant correlations between TLR4 and PPD and between TLR4 and CAL. This result agreed with a previous research that found neither CAL nor bleeding index correlate with the salivary TLR4 in periodontitis. Remarkably, there was a negative connection between the salivary TLR4 and CAL, bleeding index in gingivitis, these results suggested that salivary TLR4 could potentially represent signs of periodontitis before the bone damage.[28]

The present study is identical to previous study that found the expression levels of TLR4 in blood samples were advanced when periodontal patients were compared to controls, and relationships were found for the majority of clinical periodontal characteristics and TLRs except PPD and CAL that did not correlate with TLR4.[29] However, the result is different from Dolly *et al*.[5] which found a positive association between TLR4 and pocket depth in periodontitis, although the link is modest, and statistically, there is no important association.

About the connection coefficient between CAL and MMP9 of the current study [Table 4], there was a significant negative difference between CAL and MMP9. This may be explained as there was no active periodontitis at the time of sample collection. A study done by Hamodat *et al*.[18] found out a significant alteration in concentrations of MMP9 with PPD and CAL levels. While the previous study obtained by Jassim *et al*.[30] mentioned that MMP9 levels were directly linked with the clinical periodontal parameters (BOP and CAL). Nevertheless, significant positive correlations between MMP9 levels and CAL were found in other studies.[22,23]

Table 4 shows there were no significant correlations between PD and MMP9. The current study approved with another previous research that discovered no statistically significant variations across active MMP9 and PPD levels in rapid progression periodontitis, when compared to healthy people, slow/moderate rate of progression periodontitis and rapid progression periodontitis, the findings had higher levels of active MMP9 and latent MMP9. While in individuals with slowly/moderately developing periodontitis, there was a significant association between PPD and MMP9 activity.[25] Additionally, Alfant *et al*.[31] found there was no positive link between probing depth and MMP9 level. Meanwhile other previous study found that no statistical correlations were detected between the MMP9 level and the PPD.[25] In contrast, AlMudaris and AlRawi[32] found a positive weak (non-significant) correlation was recorded between the MMP8 with CAL among the coronary heart disease periodontitis group.

Conclusion

The current study concluded that MMP9 represents as an indicator of periodontitis and there was a significant difference between the control group and patients in level of MMP9 and TLR4, and there is a significant correlation between CAL and MMP9.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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