Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2017, 6(7): 57-66

The Profile of Tooth and Gingival Crevicular Fluid Matrix Metalloproteinase-1 in Different Dental Diseases

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ABSTRACT

Background: *Pulpitis, apical periodontitis, and chronic periodontitis are the most common dental diseases and being the leading cause of tooth loss in adults.* ***Aims:*** *To unravel the changes and the interrelation of the biochemical and immunohistochemical levels of matrix metalloproteinase-1 (MMP-1) in the gingival crevicular fluid (GCF) and teeth specimens of patients with different dental diseases. To test the influence of these changes on disease severity.* ***Materials and methods:*** *The GCF and tooth specimens were collected from 20 patients with chronic irreversible pulpitis (CIP), and similar number of patients with chronic periapical lesion (CPL), and chronic periodontitis (CP) in addition to 20 healthy controls.* ***Results:*** *Statistically significant increase were found in the mean concentration of GCF-MMP1 of the patients within the CP and CIP groups over those of CIP and CPL groups ($P<0.001$). Highly significant elevation ($P<0.001$) in the means of cell with positive expression of the MMP-1 in all patient groups compared with the mean of the control group. The highest percentages of the MMP-1 expression ($P=0.000$) above the median values were seen in CPL (13.3% vs 86.7%) followed by both CIP and CP groups (9.1% vs 90.9%). Using Receiver Operating Characteristic (ROC) curve analysis, the GCF MMP-1 was found to be an effective test in CP group at reading ≥ 0.83 pg/ml and in CPL at cut off value of ≥ 2.24 ng/ml.* ***Conclusion:*** *The MMP1 plays a crucial role in the demolition of periodontal tissue and the GCF analyses can be used as noninvasive method to unravel these changes.*

Keywords: Matrix metalloproteinase-1, Gingival crevicular fluid, Pulpitis, Periapical lesion, Chronic periodontitis

INTRODUCTION

Matrix metalloproteinase (MMPs) are a large family of calcium-dependent zinc-containing endopeptidases (*EC* 3.4.24.7), which are responsible for the tissue reconstruction and demolition of the extracellular matrix (ECM), including collagens, gelatin, matrix glycoproteins, elastin, and proteoglycan throughout organogenesis, growth, normal tissue turnover, wound healing, teeth morphogenesis, and teeth eruption [1,2]. MMPs activity in adult dental tissues are normally quite low, but rise significantly in various destructive pathological courses, like persistent inflammation and bone destructive lesions [3].

The MMP-1 was first cloned and sequenced from skin fibroblasts [4]. Since then it has been found to be produced by keratinocytes, endothelial cells, monocytes-macrophages, chondrocytes, osteoblasts, and various tumor cells. MMP-1 cleaves several ECM components but is ineffective against most basement membrane components [5,6].

Dental caries, gingivitis, pulpitis, periapical lesion as well as periodontitis are the most widespread dental diseases [7,8]. The levels of MMP-1, MMP-2, and MMP-3 are statistically elevated significantly in pulpitis and periapical lesion than in normal pulp tissue [9,10]. A meta-analysis study showed that MMP-1 1607 G1/G2 gene polymorphism was associated with CP susceptibility, as well as disease severity [11]. The major change in the biochemical constituents of the connective tissue during these dental diseases is the loss of collagens due to degradation. Periodontal disease biomarkers can be microbial factors, host response, and inflammatory mediators (e.g. cytokines, immunoglobulin, prostaglandins) well as tissue-specific biomarkers [12].

In periodontitis, the prevailing MMPs are MMP-8, MMP-9, and MMP-13 (collagenases; bone and cartilage destruction) whereas in healthy tissue, normal collagen turnover is regulated predominantly by MMP-1 or called fibroblast derived collagenases [13]. All these destructive enzymes are primarily secreted by neutrophils and responsible for type I collagen disintegration in periodontal tissue [14].

So, we aimed to measure the changes in the level of MMP-1 in GCF of a sample of Iraqi subjects and correlate these to changes in tooth expression of MMP-1 of patients suffering from periodontal diseases to test the possible use of the GCF-MMPs analysis for the severity stratification and differentiation of the dental diseases.

MATERIALS AND METHODS

The research plan was approved by the Institute Review Board at the College of Medicine, Al-Nahrain University according to the Declaration of Helsinki of 1975. Sixty patients were studied from those who were referred from the Periodontics, Orthodontics, and Oral Diagnosis Departments to Maxillo-Facial and Oral Surgery Department in Teaching Hospital of Dentistry College, University of Baghdad from September 2015 to August 2016. Each Patient was nominated for tooth extraction according to specialist`s decision who followed standard criteria for diagnosis [15]. The patient groups include; chronic irreversible pulpitis (20 patients, 9 females and 11 males), chronic periapical lesions (20 patients, 9 females and 11 males) and chronic periodontitis (20 patients, 10 females and 10 males). Twenty subjects with an apparently healthy periodontium (10 females and 10 males) were enrolled as controls with an age ranging from 27-50 years from those who attended the Outpatient Clinic at the Department of Surgery after accident, trauma. Some controls were selected from those undergoing tooth extraction as suggested by the senior in Orthodontic Department. A written consent was taken from all participants showing his/her agreement for the participation in this study. The GCF and teeth specimens were obtained from each enrolled subject. The exclusion criteria include any systemic condition that affect the host's periodontal status or that would require antibiotics such as diabetes mellitus, hypertension, heart conditions and joint replacements, or professional cleaning or periodontal manipulation within the last four weeks [16]. The GCF was collected using the white color-coded 1 µl to 5 µl calibrated volumetric microcapillary pipette (Sigma/USA). The tip of the microcapillary pipette was placed in the gingival groove of the proximal area of the selected tooth prior to extraction (unstimulated) for 5 to 20 minutes to collect a standardized volume of 4 µl GCF according to the calibration scale on the microcapillary pipette.

The GCF collected was immediately transferred to a plastic Eppendorf tube containing 400 µl phosphate buffered saline (pH-7.4) and stored at -70°C till the time of the assay using Human MMP-1 and TNF-α ELISA Kits (Mybiosource - USA). After extraction and washing of the teeth with distilled water, the teeth then immersed in 10% formalin for 72 hours for fixation and preparation of paraffin blocks. The inflammation intensity was assessed according to Accorinte et al. method [17]. Five µm-thick semi serial longitudinal sections of the teeth were mounted on clean glass slides for routine hematoxylin and eosin staining for subsequent histopathological examination. Assessment of the inflammatory cells was conducted on five separate microscopic fields for each tooth specimen at 40X magnification. The mean cell count and the severity of the inflammatory response was registered, then the slides were photographed. The other 5 µm thickness sections were mounted on positively charged microscopic slides for immunohistochemical detection of the tooth MMP-1 expression using specific monoclonal antibodies (Mybiosource, USA).

Statistical analyses

The research data were analyzed using computer program SPSS package 16.0 for Windows XP and were expressed as mean ± standard error of mean (mean ± SEM). One-way analysis of variance (ANOVA), the least significant differences, and Contingency coefficient test were used to compare between the variables. The receiver operating characteristic (ROC) curve was used to delineate the diagnostic validity of the MMP-1 by suggesting the suitable cutoff value, sensitivity, specificity, and the area under curve. The association or difference was considered, using Pearson correlation coefficient, statistically significant when $P<0.05$.

RESULTS

The mean concentrations of GCF MMP-1 in chronic irreversible pulpitis (CIP), chronic periapical lesion (CPL) and chronic periodontitis (CP) group were summarized in Table 1. ANOVA test revealed that the mean GCF-MMP-1 level in CPL group and CP groups were increased significantly ($P<0.001$) with non-significant difference in CIP group as compared with control group. Between patient groups ANOVA test illustrate considerable elevation in the mean GCF-MMP1 concentration in CP groups over those of CIP and CPL groups ($P<0.001$).

Table 1 The mean (± SEM) gingival crevicular fluid matrix metalloproteinase-1 (MMP-1) concentration among control, chronic irreversible pulpitis (CIP), chronic periapical lesion (CPL), and chronic periodontitis (CP) groups

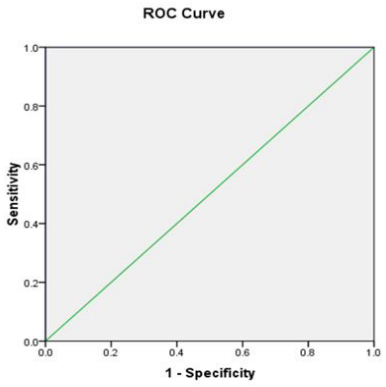
Descriptive Statistics	Studied groups			
	Controls (N=20)	CIP (N=20)	CPL (N=20)	CP (N=20)
Mean MMP-1 (ng/ml)	1.12	1.92NS	4.89a***,b***	9.41 a***,b***, c***
± SEM ‡	0.16	0.31	0.5	0.23
a ANOVA test: CIP, CPL, and CP vs. Control group: ***p<0.001, NS: Not Significant.				
b ANOVA test: CPL and CP vs. CIP: ***p<0.001				
c ANOVA test: CP vs. CPL: ***p<0.001.				
‡ SEM: standard error of mean.				

Table 2 reveals insignificant differences (P>0.05) in the mean levels of the GCF MMP-1 in males as compared to female mean values within all of the study groups.

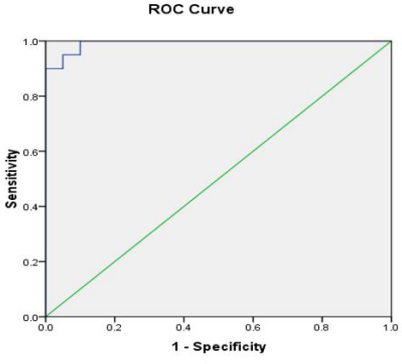
The GCF MMP-1 concentration was found to be an effective test in CP group (Figure 1A) at reading ≥ 0.83 pg/ml with 100% sensitivity and specificity of 100%, followed by its high diagnostic usefulness in CPL (Figure 1B) with sensitivity of 95% and specificity of 95% at reading ≥ 2.24 ng/ml. The lowest sensitivity in the MMP-1 level was recorded to be 60% in CIP at reading ≥ 1.78 ng/ml but with specificity of 85% (Figure 1C).

Table 2 Insignificant differences (P>0.05) in the mean levels of the GCF MMP-1 in males as compared to female mean values within all of the study groups.

Gender	Studied groups [NS]			
	Controls (N=20)	CIP (N=20)	CPL (N=20)	CP (N=20)
Male (No.)	(10)	(11)	(11)	(10)
Mean ± SEM	1.03 ± 0.023	1.57 ± 0.23	5.05 ± 0.62	9.56 ± 0.22
Female (No.)	(10)	(9)	(9)	(10)
Mean ± SEM	1.21 ± 0.22	2.34 ± 0.62	4.71 ± 0.85	9.26 ± 0.4
Concentration of MMP-1 (ng/ml); [NS] ANOVA test: Within Controls, CIP, CPL, and CP male vs. female mean values: not significant.				



(a)



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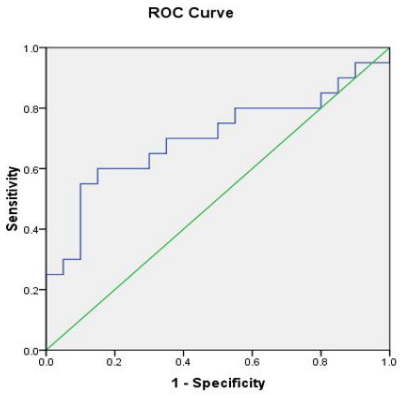

(c)

Figure 1 The receiver operating characteristic curve (ROC) for the matrix metalloproteinase 1 (MMP-1) in (a) chronic periodontitis, CP, (b) Chronic periapical lesion, CPL, (c) chronic irreversible pulpitis, CIP

Application of the Pearson correlation test revealed significant negative correlation of the GCF-MMP1 with the GCF-TNFα levels in chronic irreversible pulpitis group (r=-0.45, p<0.05). Yet, there were no significant relationships (P>0.05) between the GCF-MMP1 with the GCF-TNFα levels in other patient groups (Figure 2 and Table 2).

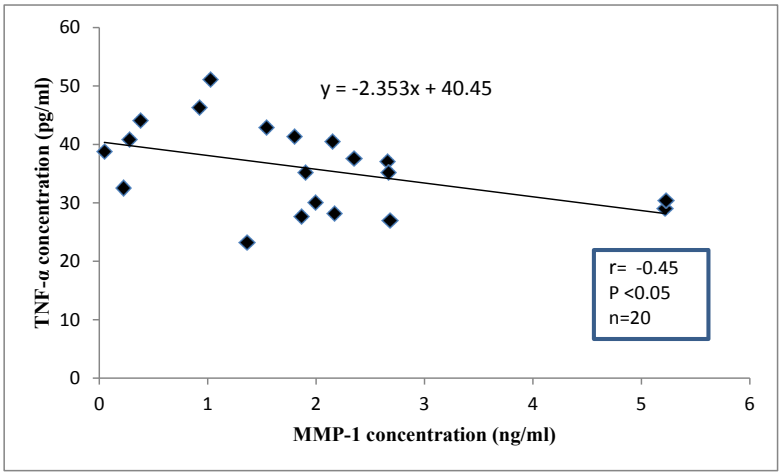

Figure 2 The correlation between the gingival crevicular fluid (GCF)-Matrix metalloproteinase-1 (MMP-1) and gingival crevicular fluid-tumor necrosis factor-α (TNF-α) levels in chronic irreversible pulpitis group

Table 3 Effects of the gender on the Mean (± SEM) concentration of matrix metalloproteinase-1 (MMP-1) among control, chronic irreversible pulpitis (CIP), chronic peri-apical lesion (CPL), and chronic periodontitis (CP) groups

Study group	Cut off value	Specificity	Sensitivity	Area under the Curve
MMP-1 in CP	0.83	100%	100%	1
MMP-1in CPL	2.24	95%	95%	0.99
MMP-1 in CIP	1.78	85%	60%	0.7

The results of the evaluation of the degree of inflammation are summarized in Figure 3. In the control group, the frequency of intensity of inflammatory reaction ranges from negative (66.7%) to weak positive (33.3%). The intensity of the inflammatory reaction for all pathological groups studied ranges between weak positive to moderate positive. The highest weak positive score was found in chronic periodontitis patient group (83.3%) followed by, chronic periapical lesion (72.2%), and chronic irreversible pulpitis (66.7%), whereas, the highest frequency of moderately positive score were seen in CIP (27.8%), followed by CPL (22.2%), then CP (16.7%). The Contingency Co-efficient (CC) test revealed highly significant differences in the inflammatory cells scores between the studied groups (CC= 0.471, P=0.002).

Table 4 The scoring frequency of the intensity or degree of inflammatory reaction in the control, chronic irreversible pulpitis (CIP), chronic periapical lesion (CPL) and chronic periodontitis (CP)

Groups	No. and Percent	Scores				Total	C.S. (*) P-value
		-ve	Weak (+)	Mod. (++)	Strong (+++)		
Control	No.	12	6	0	0	18	CC=0.471 P=0.002 HS
	% Groups	66.70%	33.30%	0.00%	0.00%	100%	
CIP	No.	0	12	5	1	18	
	% Groups	0.00%	66.70%	27.80%	5.60%	100%	
CPL	No.	0	13	4	1	18	
	% Groups	0.00%	72.20%	22.20%	5.60%	100%	
CP	No.	0	15	3	0	18	
	% Groups	0.00%	83.30%	16.70%	0.00%	100%	
Total	No.	12	46	12	2	72	
	% Groups	16.70%	63.90%	16.70%	2.80%	100%	
(*) HS: Highly Sig. at P< 0.01; Testing of random distribution are based on Contingency Coefficient test (CC).							

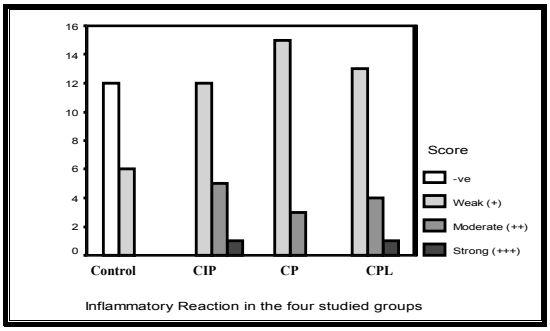

Figure 3 Cluster Bar chart of the frequency of the degree of the inflammation in the control, chronic irreversible pulpitis (CIP), chronic periapical lesion (CPL) and chronic periodontitis (CP)

The immune-histochemical MMP-1 reaction in the extracted teeth of these patients is depicted in Figure 4 (Tables 3 and 4). We found negative staining reaction in the apical area of healthy sound tooth displayed by osteoblasts (Figure 4A), a positive staining reaction for MMP-1 in CIP in the macrophages, and the endothelial cells in pulp core zone (Figure 4B). Positive staining reaction was found in the fibroblasts of CPL group (Figure 4C), and in both fibroblasts and endothelial cells of the teeth specimens of the CP group (Figure 4D).

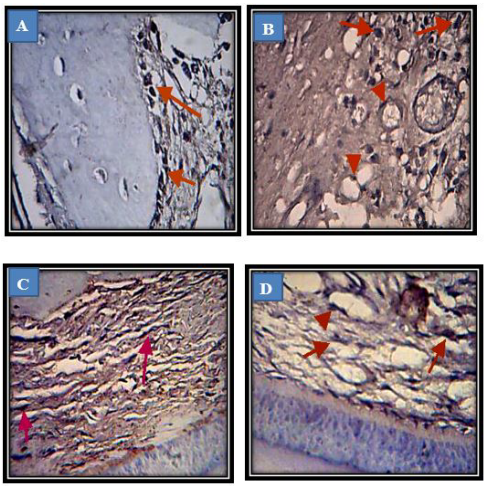

Figure 4 Immuno-histochemical view for the matrix metalloproteinase 1 (MMP-1) reaction showing :(A) negative reaction in apical region of healthy sound tooth by osteoblasts (arrows), (B) positive reaction in chronic irreversible pulpitis by macrophage (arrow), endothelial (arrow heads) in pulp core zone, (C) positive reaction in chronic periapical lesion by fibroblast (arrows), (D) positive reaction in chronic periodontitis by fibroblasts (arrow), endothelial cells (arrow head). DAB stain, magnification is 40X.

Table 5 showed high significant elevation (P<0.001) in the mean cell numbers in teeth that express positive MMP-1 staining reaction in all patient groups (CIP, CPL and CP) as compared with the mean cell counts of the control group. There was insignificant increase in the number of cell expressing positive staining reaction for MMP-1 in the CP group as compared with those of the CIP and CPL groups (P>0.05).

Using the contingency coefficient test, the highest percentage of the MMP-1 protein expression (P<0.001) above the median values as compared to the biochemical level of MMP1 in the GCF is found in the CPL group (13.3% vs. 86.7%) followed by both CIP and CP groups (9.1% vs. 90.9%, for each group), which is significant at level of P<0.01 (Table 6).

Table 5 Mean (± SEM) of cells that express matrix metalloproteinase-1 (MMP-1) in tooth tissue of control, chronic irreversible pulpitis (CIP), chronic periapical lesion (CPL), and chronic periodontitis (CP)

Descriptive statistics	Studied groups			
	Control	CIP	CPL	CP
Mean of cells with positive MMP-1 expression (cell/mm^2)	3.50	10.2***	9.20 ***	7.0 ***
± SEM	0.2	0.7	0.5	0.4
ANOVA test: CIP, PL, and CP vs Controls, ***P<0.001				

Table 6 The distribution of the interrelationship of the Immuno-histochemical and Biochemistry results of the matrix metalloproteinase 1 (MMP1 in different study groups)

Markers	Groups §	Technique	Below median	Above median	CC‡ P-value
MMP1	Control	Immunohistochemical	18	0	CC= 0.467 ***P=0.001
			62.10%	0.00%	
		Biochemistry	11	9	
			37.90%	100%	
	CIP	Immunohistochemical	17	1	CC= 0.440 **P=0.01
			63.00%	9.10%	
		Biochemistry	10	10	
			37.00%	90.90%	
	CPL	Immunohistochemical	16	2	CC= 0.482 ***P=0.001
			69.60%	13.30%	
		Biochemistry	7	13	
			30.40%	86.70%	
	CP	Immunohistochemical	17	1	CC= 0.440 **P=0.01
			63.00%	9.10%	
		Biochemistry	10	10	
			37.00%	90.90%	
‡ Contingency Coefficient (CC) test: ***P<0.001, **P<0.01					
§ CIP: chronic irreversible pulpitis, CPL: chronic periapical lesion, CP: chronic periodontitis					

DISCUSSION

Matrix metalloproteinase-1 (MMP-1) is implicated in the development of dental pathology, due to its role in the disintegration of extracellular matrix in many inflammatory diseases. The molecular mechanism beyond the pathogenesis of the dental inflammatory lesions is still un-delineated. The degradation and synthesis of ECM components in normal, healthy tissues are in constant balance, so to maintain this, collagenases are expressed at very low levels and the enzyme activity is exactly controlled [18]. The current study revealed near normal values of GCF-MMP-1 in patients with chronic irreversible pulpitis (CIP). Similar finding was observed by Shin and coworkers [7] who evaluated the correlation of the MMP-1, MMP-2, and MMP-3 with tissue destruction in acute, chronic pulpitis, periapical lesion in comparison to the control group and found insignificant differences in their levels between chronic pulpitis and control group. Yet, these findings are inconsistent with the results of Parks et al. [19] and Itagaki et al. [20] who reported that "the MMPs are present in both active and latent forms in chronically inflamed gingival tissues and gingival crevicular fluid". Active collagenase enzyme as well as gelatinizes are found in the GCF of patients with pulpitis and periodontitis in much larger amounts than in reference control subjects. Evrosimovska et al. [21] studied the concentration of collagenases (MMP-1, MMP-8 and MMP-13) in patients with chronically inflamed dental pulp

tissue and they recorded significant increase of GCF-MMP-1 levels in chronic periodontitis (CP) as compared to the control group.

The current study revealed highly significant elevation in the mean of MMP-1 in chronic periapical lesion (CPL) than control group. This finding is in agreement to the result obtained by Andonovska et al. [22] who reported a considerable increase in the concentration of MMP-1 in CPL above those of the control group. They concluded that MMP-1, MMP-8, and MMP-13 were actively participated in tissue destruction and granulated tissue formation in CPL. *In line* with these researches, Shin et al. [7] recorded significantly increased tissue level of MMP-1 in patients with acute pulpitis and periapical lesions.

Tjäderhane et al. [23] demonstrated that MMPs have a role in periapical lesion formation, because MMP inhibition significantly increases the lesion level. Accordingly, MMPs may be involved in the defensive reactions against microbes in dental pulp or periapical area. These authors confirmed that the increase of the lesion level might be due to more progressive tooth pulp infection. Apical periodontitis results from an extended inflammatory response induced by the longer exposure of periapical tissues to variety of microbial agents, evoking an immunological reaction. The inflammatory cells are the important regulators of extracellular matrix turnover and disintegration, since being the major source of reactive oxygen species and MMPs [22,24].

The metallo-proteinases can be one of the important factors in the kinetics of the periapical bone destruction and may act on the periapical bone regeneration after apitectomy and radiectomy [22]. Compared to the control group, we registered significant elevation in the mean of GCF-MMP-1 in CP. This finding was consistent with those reported by Ravi et al. [25] who demonstrated that GCF levels of MMP-1 were markedly increased in subjects with CP as compared to those of healthy controls.

Tuter et al. [26] and Soell et al. [27] reported that the clinical condition of patients with CP was significantly improved with a decrease in the GCF volume following treatment and showed levels of MMP-1 were increased in patients with chronic periodontitis before treatment ($P<0.05$) above those of the controls and after treatment. In periodontitis, the host immune response both systemically and locally in saliva and GCF plays a crucial significant role in the destruction of connective tissue and bone [28]. Periodontal destruction is most likely caused by the host cell-derived MMPs [29]. The finding of the significant increase in the mRNA of MMP-1 in the inflamed gingival tissues in CP pinpoints to the significant role of MMP-1 in the initiation of collagen degradation in periodontal diseases [30,31]. During an inflammatory response, the effectors in the immune modulation are the leukocytes that run off the basement membranes, and are equipped with MMPs enzymes which can remodel the extracellular matrix [32]. The present study, recorded no gender differences in the mean of GCF-MMP-1 in different studied groups. Although Han et al. [33] demonstrated that MMP-8, MMP-9 and MMP-13 in GCF were significantly associated with periodontitis in both genders and only the MMP-9 and MMP-13 were associated with metabolic syndrome and periodontitis in women. Our observations were in agreement with those of Cificibasi et al. [34] who found no differences in the mean age and gender allocation in the aggressive periodontitis and the control groups ($P>0.05$). Immuno-fluorometric assay (IFMA) and Enzyme-linked immunoassay (ELISA) of matrix metalloproteinase-8 (MMP-8) was carried out by Yakob and Wong [35] to differentiate between periodontitis and control subjects. Using the combination of three biomarkers, IL-1β + IL-6 + MMP-8 they were able to distinguish periodontitis from healthy periodontium with high discriminatory power (sensitivity=0.94; specificity=0.966; and AUC of 0.984). Whereas, the current research revealed that MMP-1 is an effective test in diagnosing CP at cutoff level of ≥ 0.83 ng/ml with sensitivity and specificity of 100%, followed by its diagnostic usefulness in CPL with sensitivity and specificity of 95% at cutoff reading of ≥ 2.24 ng/ml. But, MMP-1 recorded the lowest sensitivity (60%) in chronic irreversible pulpitis at cutoff level of ≥ 1.78 ng/ml. The present results elucidate the detailed analysis of expression of MMP-1 by dental cells including fibroblast, endothelial and inflammatory cells (specifically macrophage). These results revealed that the sequence of the increase in the degree of inflammation and the expression of MMP-1 in dental cells starts with high increase in CIP followed by CP and CPL groups. Evrosimovska et al. [36] detected high concentrations of collagenases in the inflamed pulp tissue with low values of collagenases in the control group. This reflects the low level of expression and activity of collagenases in physiological healthy pulp tissue, and increased level of expression in the inflamed pulp tissue. These findings confirmed the important role of MMPs in pulp tissue destruction of CP group and are similar to the results of the present study that showed significant increase in MMP-1 expression in tooth tissues of chronic irreversible pulpitis than the control group ($P<0.001$). Sakai et al. [37] revealed that macrophages are the major inflammatory

cells in dental diseases. The activated polymorphonuclear cells, macrophage, and lymphocyte are the prime source of IL-1, IL-1 and TNF-α, MMP-1 and 2 for immune-reaction in dental tissue disease. The present results showed that in periapical lesion, the periapical tissue was extremely disorganized with bone and periodontal destructions, these findings could be explained on the basis of the previous findings; first, an intense inflammatory cell infiltration presented at the periapical lesion had shared in the disorganization of the affected tissue [38]. Secondly, highly significant expression of MMP-1 may be related to resorptive factors such as cytokines IL-1β, TNF-α, parathyroid hormone, and prostaglandin E2 that lead to increase in the bone resorption and the periodontal destruction [39]. Thirdly, the initiation of the osteoclastic bone resorption, which in turn leads to the degradation of the unmineralized collagenous osteoid layer, may contribute to the presence of MMP-1 that degrades bone and collagen fibers and leads to the worsening of the periodontal disease [40-42]. Tjäderhane et al. [23] demonstrated that MMPs have a significant role in the periapical lesion formation, as the inhibition of the MMP significantly increases the severity of the periapical lesion. The MMP-1 displayed high expression level in the gingival`s epithelial and connective tissues that were affected by the periodontal disease, gingivitis and periodontitis [7]. These finding were in line with the current study findings which showed highly significant expression of MMP-1 in CP as compared to the healthy control. This reflected the importance of the MMP-1 enzyme in different stages of bone destruction observed in these periodontal diseases [43]. De Souza et al. [44] concluded that MMP-1 is produced by the stimulation of several bacteria or host factors implicated in the periodontal disease such as lipopolysaccharides, TNF-α, and IL-1. This MMP-1 has the capacity of degrading collagen type I, which is the main constitutive structural protein of the gingival connective tissue, periodontal ligament, and bone. Seabra and co researchers [45] showed that MMP-1 was expressed significantly in the CP more than those of control group and concluded that MMP-1 has a crucial role in the degradation of connective tissue and in bone loss. The present research findings of the increased levels of the MMP-1 in association with immunohistological changes in the tissue expression of the MMP-1 in different dental diseases highlighted its importance and focused on its roles in the disease prognosis. Drugs designed to control or suppress the MMP-1 expression may aid in the future eradication of these dental diseases.

CONCLUSION

A significant remarkable increase in the matrix metalloproteinase-1 (MMP-1) level in the periapical lesion and chronic periodontitis group gives additional evidence that MMP-1 play a crucial role in the periodontal tissue destruction. The MMP-1 marker showed significant association of GCF concentration with those in the tooth specimen's immuno-histochemical expression in all of the studied groups.

DECLARATIONS

Conflict of interest

The authors gave no conflicts of interest.

Acknowledgements

We are grateful to the staff of the laboratories of the Baghdad Medical City Teaching Hospital for their technical support.

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