Evaluation of salivary levels of Proteinaceous biomarkers Matrix Metalloproteinase (MMP-8) and C-Reactive Protein (CRP) in type 2 diabetic patients with periodontitis

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder of the carbohydrate, protein and fat metabolism, resulting in increased blood glucose levels. Various complications of diabetes have been described with periodontitis being added as the sixth complication of diabetes mellitus. Matrix metalloproteinase-8 (MMP-8) has been identified as major tissue-destructive enzyme in periodontal disease. MMP-8 is released from neutrophils in a latent, inactive pro form and becomes activated during periodontal inflammation by independent and/or combined actions of host-derived inflammatory mediators .C-reactive protein is a systemic marker released during the acute phase of an inflammatory response.

Subjects, materials and methods: Total samples composed of 60 participant and they divided into (20 patients un complicated type 2 diabetes with periodontitis , 20 patients non diabetic with periodontitis and 20 subjects normal control " with no sign of gingivitis or periodontitis") . Diabetes assessment was performed according to Abraham (1982).Attachment loss were assessed using periodontal disease index of Ramfjord (1959) .Un stimulated whole saliva samples were collected and chemically analyzed for quantitative measurments of salivary (MMP-8 and CRP).Blood samples were collected and then measure (HbA1c , FBS , ESR). All data were analyzed using SPSS version 20.

Results: It was found that the salivary (MMP-8, CRP) levels were lower in normal controls compared to other groups, the blood ESR level was lower in normal controls compared to other groups and there were no important differences in mean blood ESR, salivary MMP-8 and median salivary CRP between diabetic and non diabetic with periodontitis .

Conclusions: Severity of periodontitis increase with increase age , Salivary MMP-8 ,CRP and blood ESR levels were elevated in patients with periodontitis with or without diabetes , CRP and MMP-8 are considered a useful tests in predicting periodontitis ,and in type 2 diabetic patient there was a relationship between metabolic control of diabetes and severity of periodontal disease.

Key Words: Diabetes mellitus, Periodontitis, Matrix metalloproteinase-8, C-Reactive protein. (J Bagh Coll Dentistry 2013; 25(1):63-69).

INTRODUCTION

Diabetes mellitus (DM) is the most common metabolic disease worldwide. More than 90% of DM patients have type 2 diabetes, DM is the leading cause of blindness, renal failure, and lower limb amputations and consider as major risk factor for cardiovascular disease, stroke, neuropathy, and periodontitis [(1,2)]. Periodontitis is the most common complication of DM, and results from extension of the inflammatory process initiated by bacteria in the gingiva to the supporting periodontal tissues. A reciprocal relationship exists between DM and periodontal disease [(3)]. Periodontal infections, like other infections, have a significant impact on diabetic control. Conversely, DM is a significant risk factor for the development of periodontal disease and aggravates the severity of periodontal infections [(4)]. Its well known that chronic periodontitis is characterized by inflammatory cell accumulation within periodontal tissues. This situation leads to a chronic inflammation and continuous host response, resulting in tissue destruction. Four distinct pathways may be involved in this destruction: plasminogen dependent, phagocytic, osteoclastic and matrix metalloproteinase (MMP) pathway.

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Experimental evidence suggests that the most important pathway involve MMPs as active collagenase and gelatinases which are found not only in the cervicular gingival fluid but also in saliva [(5)]. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases derived from polymorphonuclear leukocytes during acute stages of periodontal disease and are the key enzymes responsible for extracellular collagen matrix degradation [(6)]. C-reactive protein is a systemic marker released during the acute phase of an inflammatory response. C-reactive protein is produced by the liver and is stimulated by circulating cytokines, such as tumor necrosis factor and interleukin-1, from local and / or systemic inflammation such as periodontal inflammation , Circulating C-reactive protein may reach saliva via gingival crevicular fluid or the salivary glands [(7,8)]. C-reactive protein has recently been shown to be measurable in saliva from periodontal patients [(9, 10)].

SUBJECTS, MATERIALS AND METHODS

The study population consisted of 20 patients with type 2 diabetes mellitus and periodontitis , 20 patients non diabetic with periodontitis and 20 subject normal control (with no sign of gingivitis or periodontitis). A prospective study was done in

Marjan General Hospital in Hilla city in Babylon and the laboratory work was done in the lab of the hospital. Diabetes assessment was performed according to Abraham [11]. Clinical examination was conducted to assess the attachment loss condition, then blood and saliva samples were taken. The periodontal examination was performed under the natural light with the patients seated on an office chair. A mouth mirror, William periodontal probe and sterilised gauzes were used for intra oral examination and the assessment of the patients (pocket depth and gingival recession to measure attachment loss). Attachment loss was assessed using periodontal disease index of [12]. All surfaces of the six Ramfjord teeth were examined. If an index tooth was missing, the nearest distal tooth was substituted for examination and the patient will be examined if he had at least 5 of 6 Ramfjord teeth. All the patients were examined by one individual. After a through clinical examination were done 10 millilitres of unstimulated whole saliva was collected and a sample of blood was taken. The collected saliva was centrifuged at 3000 r.p.m (rotation per minute) for 10 minutes and then centrifuged clear supernatant saliva kept frozen and stored at -20 °C until the whole saliva samples were collected, then the MMP-8 and CRP determination done using quantikine (Human Total MMP-8 Immunoassay) MMP-8 ELISA Kit for quantitative measurement of salivary MMP-8 and salimetrics (Salivary C-Reactive protein) ELISA Kit for quantitative measurement of salivary CRP respectively. Blood sample was taken from each individual. The whole blood was collected in sterile EDTA tubes and after complete sample collection from patients in the same day we measure HbA1c by Stanbio Glycohemoglobin Kit for quantitative determination of HbA1c in whole blood, FBS by enzymatic colorimetric method and ESR by Westergren method. Statistical analysis was done by using correlation test by the aid of the SPSS version 20 (Statistical Package for Social Sciences).

RESULTS

As shown in table (1) in this study diabetic group divided into two groups according to severity of attachment loss by consider the median of attachment loss (5.1) so less sever (<=5.1), sever (>5.1) and then make comparison as below. Mean age was obviously higher among diabetic with sever periodontitis (47.9 years) compared to those with less sever periodontitis (42.8 years), but the difference observed failed to reach the level of statistically significance. Mean blood ESR was obviously higher among diabetic with sever periodontitis (33.8 mm/hour) compared to those with less sever periodontitis (27.4 mm/hour), but the difference observed failed to reach the level of statistically significance. The median salivary CRP was obviously higher among diabetic with sever periodontitis (5562 pg/ml) compared to those with less sever periodontitis (4693 pg/ml), but the difference observed failed to reach the level of statistically significance. There was a weak and statistically insignificant positive (direct) linear correlation between attachment loss and CRP, r= 0.179, p= 0.45 [NS]. The mean salivary MMP-8 was obviously higher among diabetic with sever periodontitis (259.6 ng/ml) compared to those with less sever periodontitis (234.3 ng/ml), but the difference observed failed to reach the level of statistically significance. The mean fasting serum glucose conc. was obviously higher among diabetic with sever periodontitis (259.1mg/dl) compared to those with less sever periodontitis (184.5 mg/dl), but the difference observed failed to reach the level of statistically significance. The mean HbA1c was significantly higher (7.4 %) among diabetics with sever periodontitis compared to those with less sever periodontitis (5.9 %). Table (2) shows that non diabetic with periodontitis group divided into two groups according to severity of attachment loss by consider the median of attachment loss (5.1) so less sever (<=5.1), sever (>5.1) and then make comparison as below. There were no important or statistically significant differences in mean age, ESR and median CRP between non diabetics with sever periodontitis compared to those with less sever periodontitis. The mean MMP-8 was obviously higher (274.1 ng/ml) in those with sever periodontitis compared to those with less sever periodontitis (243.0), but the difference failed to reach the level of statistically significance. MMP-8 showed a statistically significant, moderately strong positive linear correlation (r=0.528) with attachment loss .Table (3) and figure (1) shows Receiver Operating Characteristic (ROC) test , for measuring the validity for selected parameters when used to diagnose periodontitis differentiating from healthy controls , CRP and MMP-8 were considered useful tests in predicting periodontitis (biochemical indicator of periodontal disease severity) with area under roc (1.0000, 0.999) respectively and the ESR consider the third one used for predicting periodontitis with area under ROC (0.888). Table (4) and figure (2) shows ROC test, for measuring the validity for selected parameters when used to predict DM among cases

with periodontitis , CRP , MMP-8 and ESR were not used for diagnosis of DM with area under ROC (0.627, 0.655, 0. 533) respectively.

DISCUSSION

Based on the results obtained in our study that in diabetic patients with periodontitis group, as the age of diabetic patients increase, the severity of periodontal disease increases this in accordance with result obtained by Neelima *et al.* [13], Oliver & Tervonen [14], The mean blood ESR are higher among diabetic with sever periodontitis compared to those with less sever periodontitis this in accordance with result obtained by Moder [15]. After analyzing correlations, results have shown that higher values of salivary CRP are found as periodontal disease severity increase and this in agreement with result obtained by Fernando *et al.* [16], Forner *et al.* [17]. In our study we found a correlation between increased severities of periodontitis and elevated salivary MMP-8 levels in patients with type 2 diabetes. The result of this study disagree with the result obtained by Costa *et al.* [18] who found that salivary MMP-8 concentrations were elevated regardless of periodontal inflammation in patients with type 2 diabetes mellitus. In this study, it was observed the severity of periodontal disease increase with the increase in the blood glucose level. This finding is an indicator of the need for improving oral health status among diabetic patients, Results of the present study is in accordance with previous studies by Khalid *et al.* [19]. This clinical study had found that the mean HbA1c was significantly higher among diabetics with sever periodontitis compared to those with less severe periodontitis. This means that diabetics with poor metabolic control (high HbA1c) have a higher prevalence and more extensive periodontitis than diabetics who maintain good control. Thus, metabolic control of diabetes may be an important variable in the onset and progression of periodontal disease. This is in agreement with the results obtained by Seppala & Ainamo [20]. Based on the results obtained in this present study that in non diabetic patients with periodontitis group , the severity of periodontitis not increase with or not depend on age and this disagree with other previous studies , perhaps due to the small size of study population. The results of this study disagree with the result obtained by Khansa *et al.* [21]. In this study the mean ESR not increase with or not depend on severity of periodontitis and this disagree with result obtained by Ali [22]. In this study the median salivary CRP not increase with or not depend on severity of periodontitis. In this study, it was observed that the level of MMP-8 in saliva increase with increase in severity of periodontitis. However, the difference did not reach statistical significance, perhaps due to the small size of study population. The result of this study consistent with previous studies by Lee *et al.,* and Raquel *et al.* [23, 24] they reported that the collagenase activity was positively associated with the severity of periodontal disease, and MMP-8 accounted for most of the collagenase activity in adult periodontitis patients Uitto *et al.* and Overall *et al.* [25,26]. Strong Correlation between MMP-8 and severity of periodontitis as measured by level of attachment loss, such as those described here, support the assertion that MMP-8 is a biochemical indicator of periodontal disease severity and may relate to disease activity and this in agreement with Herr *et al.* [27] who said, MMP-8 is not only indicator of disease severity but also disease activity and in agreement with result obtained by Zia *et al.* [28] who reported the same results. In this study we found that CRP was the most valid parameter in predicting periodontitis patients from normal control with sensitivity 100% and specificity 100% this is in consistent with DAiuto *et al.* [29], Zia *et al.,*[28]. The second parameter was MMP-8 with area under ROCK (0.999) with sensitivity 100% and specificity 95% this is in consistent with Mantyla *et al.* and Miller *et al.* [30,31], and in agreement with Miller *et al.* [32]. The third one is ESR with area under ROCK (0.882) and according to my Knowledge that there is no previous study that uses ESR in diagnosis of periodontitis. It is hypothesized that possibly daily episodes of a bacteremia originating from periodontal lesions are the cause for the changes in systemic markers in periodontitis. In conclusions CRP and MMP-8 are considered a useful test in predicting periodontitis, and in type 2 diabetic patients, there was a relationship between metabolic control of diabetes and severity of periodontal disease and further studies are needed with larger sample size to see the effect of having diabetes on progression of periodontal disease.

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Table 1: The difference in mean/median of selected variables between subjects with severe attachment loss (>5.1) and that with less severe among diabetics with periodontitis.

	Severe Attachment loss (>5.1)		
	Less severe(<=5.1) (n=11)	Severe (>5.1) (n=9)	P value
Age in years			0.1[NS]
Range	(31 - 58)	(42 - 55)	
Mean	42.8	47.9	
SD	7.7	4.8	
SE	2.3	1.6	
r=0.378 P=0.1[NS]			
Blood ESR (mm/hour)			0.41[NS]
Range	(1 - 48)	(13 - 62)	
Mean	27.4	33.8	
SD	17.7	15.7	
SE	5.3	5.2	
r=0.023 P=0.92[NS]			
CRP (C-reactive protein) pg/ml			0.13[NS]
Range	(3144 - 6487)	(3384 - 33236)	
Median	4693	5562	
Interquartile range	(3384 - 5864)	(4148 - 8493)	
r=0.179 P=0.45[NS]			
MMP-8 (Matrix metallo proteinase-8) ng/ml			0.24[NS]
Range	(118 - 299)	(221 - 322)	
Mean	234.3	259.6	
SD	51.2	39.5	
SE	15.4	13.2	
r=0.186 P=0.43[NS]			
Fasting serum glucose conc. (mg/dl)			0.1[NS]
Range	(110 - 365)	(135 - 389)	
Mean	184.5	259.1	
SD	87.4	103.0	
SE	26.4	34.3	
r=0.42 P=0.07[NS]			
Hb A1c % (glycosylated Hb)			
Range	(4.2 - 7.9)	(5.6 - 8.9)	
Mean	5.9	7.4	
SD	1.4	1.0	
SE	0.4	0.3	
r=0.43 P=0.06[NS]			

Table 2: The difference in mean/median of selected variables between subjects with severe attachment loss (>5.1) and that with less severe among non-diabetics with periodontitis.

	Severe Attachment loss (>5.1)		
	Less severe (<=5.1) (n=10)	**Severe (>5.1) (n=10)**	**P value**
Age in years			0.66[NS]
Range	(35 - 55)	(30 - 55)	
Mean	45.1	46.6	
SD	5.9	8.8	
SE	1.9	2.8	
r=0.032 P=0.89[NS]			
Blood ESR (mm/hour)			0.86[NS]
Range	(10 - 60)	(12 - 80)	
Mean	34.5	36.0	
SD	14.2	21.7	
SE	4.5	6.9	
r=0.157 P=0.51[NS]			
CRP (C-reactive protein) pg/ml			0.62[NS]
Range	(3144 - 37221)	(3886 - 44521)	
Median	4696.5	4834.5	
Interquartile range	(4148 - 8145)	(4417 - 7803)	
r=0.287 P=0.22[NS]			
MMP-8 (Matrix metallo proteinase-8) ng/ml			0.14[NS]
Range	(199 - 299)	(217 - 369)	
Mean	243.0	274.1	
SD	34.2	53.5	
SE	10.8	16.9	
r=0.528 P=0.017			

Table 3: ROC area measuring the validity for selected parameters when used to diagnose periodontitis differentiating it from healthy controls.

	ROC area	P
CRP (C-reactive protein) pg/ml	1.000	<0.001
MMP-8 (Matrix metallo proteinase-8) ng/ml	.999	<0.001
Blood ESR (mm/hour)	.882	<0.001

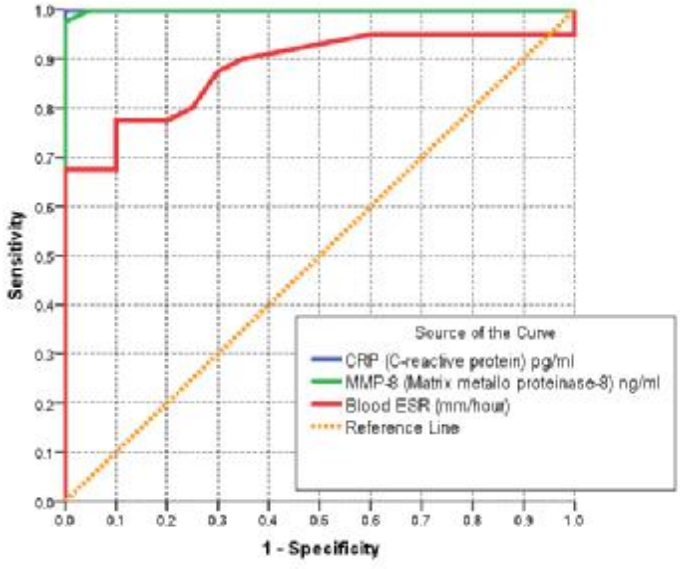

Figure 1: ROC curve showing the trade-off between sensitivity and 1-specificity for selected parameters when used to diagnose periodontitis differentiating it from healthy controls.

Table 4: ROC area measuring the validity for selected parameters when used to predict DM among cases with periodontitis.

CRP (C-reactive protein) pg/ml	.627	0.17[NS]
MMP-8 (Matrix metallo proteinase-8) ng/ml	.655	0.09[NS]
Blood ESR (mm/hour)	.533	0.72[NS]

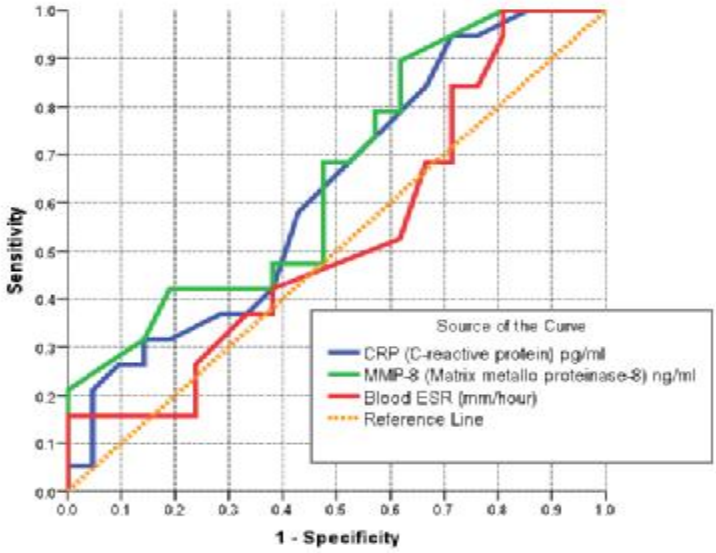

Figure 2: ROC curve showing the trade-off between sensitivity and 1-specificity for selected parameters when used to predict DM among cases with periodontitis.