

Salivary Matrix Metalloproteinase (MMP-8) in Relation to Periodontal Health Status among a Group of Patients with Acquired Coronary Heart Disease

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Abstract: Background: Many studies have investigated relation between oral health and periodontal diseases with coronary heart diseases. Although the evidence linking periodontal diseases with coronary heart diseases is not yet conclusive, recent evidence raised the support to the role of periodontal diseases in the initiation and progression of coronary heart disease. Aim of study: This study was conducted to measure salivary matrix metalloproteinase - 8, in relation to periodontal pocket depth and clinical attachment loss, among a group of patients with coronary heart disease in comparison with control group. Materials and methods: Sixty subjects, aged 45-50 years old, they were seeking treatment for chest pain. They were divided into study group (30 patient), were diagnosed to be a candidates percutaneous coronary interventions (PCI) group, and a control group (30 subject), who scored a negative result in the tread mill test, and was no need for a cardiac angiography. Probing pocket depth (PPD) and clinical attachment level (CAL), were measured according to Lindhe et al. (1999). Unstimulated saliva was collected from all subjects, according to Navazesh and Kumar, (2008), to analyses MMP-8. Result: A high mean value of CAL for the PCI group than the control group with statistically highly significant difference. High mean value of PPD for the PCI group than the control group, a statistically, non-significant was found. Salivary MMP-8 level was less in the PCI group than in the control group, with statistically no significant difference between groups. A positive correlation between MMP-8 with PPD. and CAL in the PCI groups. However, statistically non-significant ($P > 0.05$). Conclusion: Although periodontal diseases revealed higher percentage of occurrence among a group of patients with coronary heart disease, salivary MMP-8 were found to have little effects on the periodontal status of the study group.

Keywords: Salivary Matrix Metalloproteinase (MMP-8), Periodontal pocket depth, Acquired coronary heart disease.

1. Introduction

Coronary artery disease is caused by atherosclerosis of the coronary arteries that leads to a restriction of blood flow to the heart.¹ In coronary heart disease (CHD), many risk factors, such as family history, obesity, diabetes mellitus, hypertension, dyslipidemia, and smoking have been linked to the development of atherosclerosis.² Chronic oral diseases may increase the risk of CHD and may be an unconventional risk factor for CHD.³

Coronary angiography a diagnostic technique in which a radio contrast is injected directly into the coronary arteries, allowing visualization and quantification of stenosis and/or obstruction.⁴

Periodontal diseases elicits an inflammatory response.⁵ There is controversial scientific evidence regarding the association between chronic periodontitis and CAD. The mechanisms through which periodontal diseases could affect cardiovascular health include direct contamination by bacteria, immune-mediated injuries, and inflammation.⁶

Matrix metalloproteinase (MMP-8) is currently regarded among the most important key biomarkers of inflammation.⁷ MMP-8 can initiate the digestion of type I collagen, which is the major structural element and load-bearing molecule that provides tensile strength to the fibrous cap of an

atherosclerotic lesion.⁸ In addition to the inflammation caused by atherosclerosis, the sources of MMP-8 found in serum by the host response to the insult of periodontal pathogens involves local increases of MMP-8 level, which may most probably leak to circulation through inflamed periodontal tissues.⁹

2. Materials & Methods

Subjects

Sixty (60) subjects, males only, aged 45-50 years old were included in this study, they were seeking treatment for chest pain. The sample collection was done in Ibn-AL Baytar center for cardiac surgical treatments in Baghdad, Iraq. The subjects were divided into study group (30 patient), and a control group (30 subject), as following:

- 1) Study group: Percutaneous coronary interventions (PCI) group, patients who were diagnosed to be a PCI candidates, after going through the diagnostic cardiac angiography.
- 2) Control group: patients who scored a negative result in the tread mill test, and in no need for a cardiac angiography.

Inclusion criteria

- 1) Patients suffering from chest pain, who is either:
 - Free from chronic systemic diseases.

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- Or had a history of hypertension (diagnosed for previous 1 year at least). Allowed drugs includes only Angiotensin II Receptor Blockers (ARBs).

Exclusion criteria included:

- 1) Patient who had a history of chronic systemic diseases, other than hypertension, such as diabetes mellitus, rheumatoid arthritis, etc. which have a known association with the periodontal diseases.
- 2) Patients with a previous history of PCI and stent placement.
- 3) Smoker.
- 4) Medication (anti-inflammatory or antimicrobial therapy) within previous 3 months.
- 5) Periodontal treatment within previous 3 months.

Saliva collection

Unstimulated whole saliva samples were collected at a fixed collection time (8-11 a.m.). The patient was advised to quit the intake of any food or beverage (water excluded) one hour before the test session. The subjects were advised to rinse their mouth with water and then to relax for five minutes. The patients were asked to swallow before the beginning of the collection and to lean their head forward over the test tube and minimize mouth movements as possible, and allow saliva to drain into the tube for a 5 minutes (Navazesh and Kumar, 2008).¹⁰

Assessment of clinical attachment level (CAL):

Clinical attachment level is defined as the distance from the cemento-enamel junction (CEJ) to the location of the inserted probe tip (bottom of the gingival crevice or pocket) (Lindhe et al., 1999)¹¹. The measurements were made at four surfaces of each tooth except third molar. The CAL was measured indirectly by subtracting the distance from the gingival margin to the cemento-enamel junction from PPD. In some cases where there was gingival recession, attachment level was measured either directly or by adding the distance from the gingival margin to the CEJ to the PPD. The level of the CEJ could be determined by feeling it with a probe. Assessment of severity was done according to the criteria illustrated in table (1), (Armitage, 1999)¹²:

In some situations, the tooth was excluded, because the CEJ was totally obliterated by:

- 1) Full crown coverage.
- 2) disto-occlusal, mesio-occlusal, buccal/labial, palatal/lingual, or MOD filling were extended below the CEJ.
- 3) Badly decayed tooth, where the caries extended distally, mesially, buccally/labially, palatally/lingually below the CEJ.

Assessment of probing pocket depth (PPD):

Probing pocket depth is defined as the distance from gingival margin to the most apical penetration of the periodontal probe inserted into the gingival crevice or pocket without any force or pressure. The sites of measurement was mid-buccal line, mid palatal/lingual line, mesio-buccal, and disto-buccal line angles (Lindhe et al., 1999)¹¹. The PPD measurement has been performed by using the Marquis periodontal probe. Classification the severity of probing pocket depth illustrated in table (2), (ADA, 1996)¹³:

Salivary biochemical analysis:

Salivary MMP-8 analysis:

The concentration of salivary Matrix Metalloproteinase 8/neutrophil collagenase (MMP-8) was determined by using the supernatant salivary samples with mean of Enzyme Linked Immuno-Sorbent Assay (ELISA). ELISA Kit (96-wells) for quantitative determination of salivary MMP-8 of SHANGHAI YEHUA biological technology Co.

Principle of reaction:

This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Matrix metalloproteinase 8/Neutrophil collagenase (MMP-8). Add Matrix metalloproteinase 8/Neutrophil collagenase (MMP-8) to wells that are pre-coated with Matrix metalloproteinase 8/Neutrophil collagenase (MMP-8) monoclonal antibody and then incubate. After incubation, add anti MMP-8 antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, then add substrate A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of solution and the concentration of Human Matrix metalloproteinase 8/Neutrophil collagenase (MMP-8) are positively correlated.

Data processing and statistical analysis:

Entering the data was done by personal computer. Statistical analysis was done by using correlation test by the aid of the SPSS version 21 (Statistical Package for Social Sciences).

3. Result

Periodontal health status:

Clinical attachment level (CAL):

The mean values and standard deviations of the clinical attachment level for the study and control groups are shown in Table (3). From the table, it was found that the mean value of CAL for the PCI group was higher than that for the control group with statistically highly significant difference ($P < 0.01$).

Probing pocket depth (PPD):

The mean values and standard deviations of the probing pocket depth for the study and control groups are shown in Table (4). From the Table, it was found that the mean value of PPD for the PCI group was higher than that for the control group, however, a statistically non-significant difference was found ($P > 0.05$).

Salivary matrix metalloproteinase 8 (MMP-8):

Table (5) illustrates the mean values and standard deviations of salivary MMP-8 between study and control groups. It was found that salivary MMP-8 level was slightly less in the PCI group than the control. However, statistically no significant difference was recorded between groups ($P > 0.05$).

Table (6) demonstrates the correlations between MMP-8 with mean percentage of PPD and CAL, among study and control groups. A positive weak correlation was recorded between the MMP-8 with CAL among PCI group, while a negative weak correlation is noted among the control group,

however, statistically no significant ($P>0.05$). The relation between MMP-8 with PPD was positive weak non-significant in the PCI, and control group ($P>0.05$).

4. Discussion

The sample

The study sample consists of males who they were seeking treatment for chest pain, their ages were between 45-50 years. The selection of this age group may be attributed to the fact that, atherosclerosis starts early in life, since disease progression is usually slow, clinical symptoms with or without hospitalization is rare before 45 years of age.¹⁴

The sample was non-smokers and non-diabetic, and not take any medications in the last three months to exclude their effects on the periodontal condition except the effect of hypertension drug.

Periodontal condition and cardiovascular diseases

The findings of this study, recorded that, mean value of clinical attachment loss (CAL) and periodontal pocket depth (PPD) for the cardiovascular diseases group higher than that for the control group. This may be attributed to the fact that, periodontitis (PD) is a bacterially-induced, localized chronic inflammatory disease destroying both the connective tissue and the supporting bone of the teeth.¹⁵ In addition to that, in the general population, severe forms of the disease demonstrate a prevalence of almost 5%; whereas, initial epidemiological evidence suggests an association between PD and coronary artery disease (CAD),¹⁵ regarding the same fact, fibroatheroma is diagnosed in patients aged 40 years and over, in the similar age group where periodontitis is diagnosed in more than 50% of patients.¹⁶ This results also agree with findings of Blaizot and colleagues explored 215 epidemiological studies and found the risk of developing CVD significantly higher in subjects with periodontal disease compared to those without periodontal disease ($P < 0.0001$).¹⁷

A statistically highly significant mean value of clinical attachment loss was recorded in the groups with cardiovascular diseases, these results of this study, similar to the study recorded by Samani and associates further showed imperative relationship between mean attachment loss and CAD in the patients who lost more than 10 teeth.¹⁸ In addition to that, this was somewhat similar to the study results of Ramesh and coworkers who reported PD in 11 patients of the acute coronary syndrome group and 10 patients in the healthy group.¹⁹ Rutger Persson and associates also suggested that patients who at routine dental visits demonstrate evidence of bone loss around several teeth can predictably be identified as being at risk for future CAD.²⁰

Biochemical analysis of saliva

Saliva is a complex secretion whose components exert a well-documented role in health and disease, it is emerging as a viable alternative to blood sampling.²¹

Similar to other biological systems, the salivary MMPs system includes various molecules and enzymes.²² In this study, unstimulated saliva was collected, as it more convenient and easier to obtain the required and adequate quantity of saliva. The biochemical analysis results of this

study revealed slightly less mean value of salivary MMP-8 among study group than the control group with non-significant difference between the two groups.

In spite of the findings of the current study, MMP-8 mean value in groups with cardiovascular diseases slightly lesser than the level of control group, however, these findings may be attributed to that, MMP-8 is catalytically the most competent proteinase to initiate type I collagen and extracellular matrix degradation associated with periodontal destruction. Regarding cardiovascular diseases, pathologically MMP-8 has been implicated in atherosclerotic plaque destabilization and rupture probably through its proteolytic ability to thin the protecting collagenous fibrous cap lining coronary and other arteries.²³

Nevertheless, one can perceive parallelisms between periodontal tissue demolition and CVD as both mediated by a similar pathway via MMPs. In actual fact, there is increasing evidence that inhibition of MMPs, already shown to be effective for inhibition of periodontal attachment loss, can also inhibit the development of cardiac failure.²⁴

This fact supported with the findings in the current study, the positive correlation between MMP-8 mean value with periodontal disease (periodontal pocket depth and clinical attachment level), among a group with cardiovascular diseases this may be related with the mechanism of action that, during the initiation and course of inflammatory responses in periodontitis and cardiovascular diseases, proinflammatory mediators including especially MMP-8 are up-regulated not only in affected tissues but also in the secreted, disease affected, oral fluids (gingival crevicular fluid, peri-implant sulcular fluid, mouth rinse and saliva) as well as in serum and plasma.²³

Besides, the result of present research give a positive relations of periodontal diseases index with the salivary MMP-8, among a group with coronary artery disease, although statistically were not significant, this attributes to function of certain salivary protein, may have both protective and detrimental properties.²⁵ Thus, salivary proteins can be known as "Double edged" swords. Functions of salivary proteins may depend on the molecule's location or site of action.²⁶

Results of this study also revealed that oral health status may have an impact on the general health of group with cardiovascular disease, in addition to that, these group of people need additional attention with a special oral as well as general health preventive program also a special dental health care centers should be offered. So it becomes the duty of the dentist to include cardiovascular disease in differential diagnosis of oral health condition, to avoid the needless delay in the treatment of this disease.

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Table 1: Severity of clinical attachment level

Score	Severity of clinical attachment level
1 – 2 mm	Mild
3 – 4 mm	Moderate
≥ 5 mm	Severe

Table 2: Severity of probing pocket depth

Score	Severity of probing pocket depth
4-5 mm	Mild
6-7 mm	Moderate
>7 mm	Severe

Table 3: Clinical attachment level mean and standard deviation (M±SD) among groups of patients with chest pain

Group	Mean	±SD	F (T)	df	P. value
PCI	2.874	1.040	17.662	2	0.000
Control	1.760	0.546			

Table 4: Probing pocket depth mean and standard deviation (M±SD) among groups of patients with chest pain

Group	Mean	±SD	F (T)	df	P. value
PCI	3.211	1.819	2.258	2	0.111
Control	2.167	2.065			

Table 5: Matrix metalloproteinase- 8 mean and standard deviation (M±SD) among groups of patients with chest pain

Group	Mean	±SD	F (T)	df	p. value
PCI	2.004	0.737	2.800	2	0.066
Control	2.331	1.236			

Table 6: Correlation coefficients between matrix metalloproteinase- 8 with probing pocket depth and clinical attachment level, among groups of patients with chest pain.

Variable	PCI		Control	
	r	p	r	p
PPD	0.115	0.547	0.102	0.592
CAL	0.251	0.181	-0.130	0.494