

Salivary Matrix Metalloproteinase-8 (MMP-8) in Relation to Periodontal Health Status Among a Group of Hypertensive Patients

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ABSTRACT

Background: Hypertension is probably the most important public health problem around the world. People with periodontal disease may be at greater risk of hypertension. The inflammatory effects of periodontal disease help to promote endothelial dysfunction in arteries which may lead to changes in blood pressure. Salivary MMP-8 has been associated with both periodontal disease and prevalent hypertension.

Aim of study: This study was conducted to measure salivary matrix metalloproteinase - 8, in relation to periodontal health condition among a group of patients with hypertension in comparison with control group.

Materials and methods: Ninety subjects, aged 45-50 years old were included in this study, seeking treatment for chest pain in Ibn-ALBaytar center for cardiac surgical treatments in Baghdad, Iraq. The subjects were divided into study group (45 patient) who were diagnosed to be a hypertensive patient, and a control group (45 subject), with no hypertension. Plaque status was evaluated according to the Silness and Loe, probing pocket depth and clinical attachment level. Unstimulated saliva was collected from all subjects to analyses MMP-8. **Result:** A high mean value of plaque index, clinical attachment level and probing pocket depth for the study group than the control group with statistically no significant difference. In addition to that, a significant positive correlation between the plaque index and the clinical attachment level among both groups. Salivary MMP-8 level showed a higher level in the study group than in the control group, with statistically significant difference between groups, and a significant positive correlation was detected between salivary MMP-8 with plaque index, among study group.

Conclusions: Higher percentage of periodontal diseases was found among patients with blood hypertension. In addition, high level of salivary MMP-8 is potentially associated with periodontal status of the study group.

Key words: Salivary Matrix Metalloproteinase (MMP-8), Probing pocket depth, blood hypertension. (J Bagh Coll Dentistry 2018; 30(3): 48-53)

INTRODUCTION

Hypertension (HT), also known as high blood pressure (HBP), are a long term medical and clinical conditions in which the blood pressure in the arteries is persistently elevated⁽¹⁾. High blood pressure usually does not cause symptoms⁽²⁾. Long term high blood pressure is a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss, and chronic kidney disease^(3,4). High blood pressure affects between 16 and 37% of the population globally⁽⁵⁾. Data from 2011 to 2014 demonstrated that 46 percent of adults 18 years and older in the United States had hypertension⁽⁶⁾. Dentists have a rare opportunity to detect the cases of hypertension. It is a professional responsibility of dental clinician to inform the patient of their hypertensive state and to offer medical advice, including appropriate referrals. Antihypertensive drugs can often cause side-effects, such as xerostomia, gingival overgrowth, salivary gland swelling or pain, lichenoid drug reactions, taste sense alteration⁽⁷⁾.

However, dental disease has often oral manifestation of acute, chronic, and systemic disease. Afflictions such as heart disease, diabetes, stroke, hypertension, multiple sclerosis, and HIV/AIDS often can be discovered during a routine visit to the dentist⁽⁸⁾.

Periodontitis is one of the most chronic oral infectious diseases. The mouth is an ideal breeding area for bacteria and those affected by periodontal disease are at increased risk, for potentially fatal bacteria entering the bloodstream via infected oral tissue. Those suffering from periodontitis are highly susceptible to sever and major health issues such as premature births and low birth-weight babies, anorexia, vascular and heart disease^(8,9). People with periodontal disease may be at greater risk of heart attack or stroke. Recent studies have shown that the inflammatory effects of periodontal disease help to promote blood clot formation in arteries⁽¹⁰⁾. Several inflammatory mediators are present, and can be reproducibly measured in saliva⁽¹¹⁾. Salivary MMP-8 has been associated with prevalent hypertension and coronary artery disease. In addition, salivary levels of MMP-8 are increased in acute coronary syndromes⁽¹²⁾. Salivary levels of potential mediators of atherosclerosis could be used as biomarkers for subclinical cardiovascular disease. Salivary measures may not only represent an easy to use,

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noninvasive test for cardiovascular risk, but could potentially also expand the possibility to assess novel biomarkers, whose systemic levels may be of questionable value⁽¹³⁾.

Since periodontitis is a chronic inflammatory infection, the suggestion of periodontitis as a risk factor for hypertension has begun lately. Nowadays, there is more evidences supports periodontal disease as an independent risk factor for cardiovascular diseases⁽¹⁴⁾. The renin-angiotensin-aldosterone system (RAAS) is a coordinated hormonal cascade that controls cardiovascular, renal, and adrenal function by governing body fluid and electrolyte balance as well as arterial pressure. Angiotensin II (ang II) is the principal effector peptide of the RAAS. Through its binding to the ang II type 1 (AT1) receptor, it mediates a range of processes, including vasoconstriction, aldosterone, and vasopressin release, sodium and water retention, and sympathetic activation. These, in turn, can lead to the development of hypertension and to the hemodynamic alterations of congestive heart failure⁽¹⁵⁾.

Angiotensin II receptor blockers (ARB) act by selectively blocking the binding of ang II to the AT1 receptor⁽¹⁶⁾.

This study was conducted to measure salivary matrix metalloproteinase -8, in relation to periodontal health condition among a group of patients with hypertension in comparison with control group.

MATERIALS, AND METHODS

Subjects:

Ninety 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 during the period between the mid of December 2016 and the mid of April 2017 in Ibn-AL Baytar center for cardiac surgical treatments in Baghdad, Iraq.

The subjects were divided into study group (45 patient), and a control group (45 subject), as following:

- 1-Study group: patients who were diagnosed to be hypertensive patients, and use antihypertensive drug.
- 2- Control group: patients were collected from treadmill test room, free from hypertension, which scored a negative result in the blood pressure test, and in no need for any antihypertensive drug.

Inclusion criteria:

- Patients suffering from chest pain, who are:
- Free from chronic systemic diseases.

-With a history of hypertension (diagnosed for previous 1 year at least), allowed drugs includes only Angiotensin II Receptor Blockers (ARB):

- Valsartan 80-320 mg
- Telmisartan 20-80 mg
- Losartan 25-100 mg
- Irbesartan 150-300 mg
- Azilsartan 40-80 mg
- Olmesartan 20-40 mg
- Candesartan 8-32 mg
- Eprosartan 400-800mg

Exclusion criteria:

1. Patient who had a history of chronic systemic diseases, other than hypertension, including diabetes mellitus, rheumatoid arthritis, which have a known association with the oral inflammation.
2. Patients with a previous history of percutaneous coronary interventions and stent placement.
3. Patients who are smokers.
4. Patient with medication (anti-inflammatory or antimicrobial therapy) within previous 3 months.
5. Patient who had periodontal treatment within previous 3 months.

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⁽¹⁷⁾.

Clinical parameters examination was done after salivary sample collection. Dental plaque assessment was done by using PLI system⁽¹⁸⁾ (Table 1), and was performed on six index teeth as follow: upper right first molar, upper right lateral incisor, upper left first premolar, lower left first molar, lower left lateral incisor, lower right first premolar.

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. By examining four surfaces of all teeth except the third molar for the probing pocket depth index by using the Marquis periodontal probe. The sites of measurement were mid-buccal line, mid palatal/lingual line, mesio-buccal, and disto-buccal line angles⁽¹⁹⁾.

The severity of probing pocket depth as following⁽²⁰⁾:

4-5 mm represents the mild type.

6-7 mm represents the Moderate type.

>7 mm represents the Severe type.

Clinical attachment level (CAL) 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). 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 as following⁽¹⁹⁾:

1 – 2 mm represents the mild type.

3– 4 mm represents the moderate type.

≥ 5 mm represents the severe type.

The concentration of salivary MMP-8/neutrophil collagenase (MMP-8) was determined by using the supernatant salivary samples by utilizing Enzyme Linked Immune-Sorbent Assay (ELISA). ELISA Kit (96-wells) for quantitative determination of salivary MMP-8 purchased from SHANGHAI YEHUA biological technology Co. This kit uses ELISA based on biotin double antibody sandwich technology to assay Human MMP-8/Neutrophil collagenase (MMP-8). Salivary samples were added to wells that are pre-coated with MMP-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. Unbound enzymes were removed after incubation by washing. Then substrate (chromogenic reagent) A and B were added, the solution will turn blue and change to yellow with the effect of acid. The optical density of MMP-8 in the samples was measured by using Spectrophotometer. Statistical analysis was determined by T-test and Pearson's correlation coefficient test using version 18 (Statistical Package for Social Sciences SPSS).

RESULTS:

Table 1 illustrates the mean value and standard deviation of PLI among study and control group. The mean values of plaque status were higher among the hypertensive patients than the non-hypertensive patients. However, the

difference between the two groups was not significant.

Table 1: Plaque index mean and standard deviation among patients with and without hypertension.

Group	Mean	±SD	P-value
With hypertension	1.603	0.464	0.624
Without hypertension	1.553	0.496	

The mean values and standard deviations of the CAL for the study and control groups are shown in Table 2. From the table, it was found that the mean value of CAL was statistically not different between the patients with hypertension and non-hypertensive.

Table 2: Clinical attachment level mean and standard deviation among patients with and without hypertension.

Group	Mean	±SD	P-value
With hypertension	2.275	0.880	0.673
Without hypertension	2.196	0.877	

The mean values and standard deviations of the PPD for the study and control groups are shown in Table 3. From the table, it was found that, the patients with hypertension had higher mean value of PPD than non-hypertensive patients. However, the difference was found to be not significant.

Table 3: Probing pocket depth mean and standard deviation among patients with and without hypertension.

Group	Mean	±SD	P-value
With hypertension	2.872	1.947	0.468
Without hypertension	2.566	1.990	

Table 4 illustrates the mean values and standard deviations of salivary MMP-8 between study and control groups. It was found that hypertensive patients showed a statistically ($p < 0.05$) higher mean value of salivary MMP-8 level than that observed for the non-hypertensive group.

Table 4: Matrix metalloproteinase- 8 mean and standard deviation among patients with and without hypertension.

Group	Mean	±SD	P-value
With hypertension	2.648	1.477	0.030*
Without hypertension	2.020	1.104	

*significant $p < 0.05$

Table 5 demonstrates the correlation coefficients between PLI, with PPD and CAL, among groups of patients with and without hypertension. The

correlations between mean scores of PLI and PPD, showed no significant relation in both groups. Regarding CAL, a different pattern was noted, a positive significant correlation between the PLI with CAL among both groups.

Table 5: Correlation coefficients between plaque index, with probing pocket depth and clinical attachment level, among groups of patients with and without hypertension.

Variable	With hypertension		Without hypertension	
	r	p	r	p
PPD	0.004	0.980	0.299	0.068
CAL	0.538	0.000*	0.724	0.000*

* significant $p < 0.05$

Table 6 shows the correlation coefficients between mean level of salivary MMP-8 with PLI, PPD, and CAL, among the study and control groups. No significant correlation was detected between salivary MMP-8 with PPD and CAL, while the relation between MMP-8 with PLI among hypertensive patients, recorded a significant coefficients.

Table 6: Correlation coefficients between Matrix metalloproteinase-8 with plaque index, probing pocket depth and clinical attachment level, among patients with and without hypertension.

Variable	With hypertension		Without hypertension	
	r	p	r	p
PLI	0.296	0.033*	0.042	0.801
PPD	0.073	0.608	0.04	0.813
CAL	0.133	0.349	0.081	0.630

* significant $p < 0.05$

DISCUSSION

The study sample consisted of males who were seeking treatment for chest pain, and severe headache, their ages ranged between 45-50 years. The selection of this age group may be attributed to the fact that, long term high blood pressure, is a major risk factor for coronary artery disease and atherosclerosis starts early in life, since disease progression is usually slow, clinical symptoms with or without hospitalization is rare before 40 years of age⁽²¹⁾. In the current study, plaque index by Silness and Loe⁽¹⁸⁾ was used for assessment of dental plaque thickness, this index widely used due to validity, their ease, and feasibility⁽²²⁾.

The results of the current study showed that the mean value of the PLI was higher among hypertensive group as compared to control

group. These results may be due to oral health negligence in the study groups and this could be attributed also to lack of motivation about dental plaque control. In addition to the previous causes, the finding of this study may also be linked to the fact that dental plaque bacteria are considered one of the risk factors for atherosclerosis development⁽²³⁾. Circulating microorganisms or their products may promote pathogenesis and enhance local inflammatory changes in vessel walls that may promote clotting and clot formation which lead to hypertension⁽²⁴⁾.

The findings of this study recorded that, the mean values of CAL and PPD, for patients suffering from blood hypertension, were higher than that for the healthy subjects. These results may be explained by the well-known association between hypertension and periodontitis which shares common risk factors, such as, stress, increased age, and socioeconomic factors⁽²⁵⁾. A variety of studies have found various results showing an association between hypertension and periodontitis. These studies documented that hypertensive subjects exhibited a more detrimental periodontal status⁽²⁶⁻²⁸⁾.

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⁽²⁹⁾. Similar to other biological systems, the salivary system includes various molecules and enzymes⁽³⁰⁾, in this study, un stimulated saliva was collected, as it is more convenient and easier to obtain the required and adequate quantity of saliva. The biochemical analysis results of this study revealed more mean value of salivary MMP-8 among the hypertensive group than the control group with statistically significant difference, this may be related to the general health of the study group who are having health problems regarding their coronary atherosclerotic condition.

Current findings agree to some extent with earlier studies in which the respective MMP-8 was higher in hypertensive patients than in healthy subjects⁽³¹⁻³³⁾. Results also showed positive correlations between MMP-8 mean value with periodontal disease (plaque index, probing pocket depth, and clinical attachment level) among the group complain from blood hypertension, these results may be explained by the fact that MMP-8 is catalytically the most competent proteinase to initiate type I collagen and extracellular matrix degradation associated with periodontal destruction⁽³⁴⁾. Regarding cardiovascular diseases, MMP-8 has been implicated in atherosclerotic plaque

destabilization and rupture pathologically through its proteolytic ability to thin the protecting collagenous fibrous cap lining coronary and other arteries⁽³⁵⁾. Through these two facts one can perceive parallelisms between periodontal tissue destruction and blood hypertension as both mediated by a similar pathway via MMPs. In addition to that, increased MMP- 8 level promotes vascular smooth muscle cells migration and proliferation and endothelial dysfunction, thus resulting in increased cardiovascular stiffness and hypertrophy, promotes long-lasting cardiovascular structural and functional alterations which may contribute to sustained hypertension and its complications⁽³⁶⁾. This biomarker also differentiated between patients with or without hypertension, being significantly higher in hypertensive patients. These findings illustrate the importance to consider the effect of systemic diseases when analyzing oral condition. In addition to that, these groups of people need additional attention with a special oral and general health preventive program also a special dental health care centers should be offered.

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الخلاصة

الخلفية: يعد ارتفاع ضغط الدم على الاغلب من اهم مشاكل الصحة العامة في الدول المتطورة والنامية . انه منتشر, عديم الاعراض, ممكن تشخيصه بسهولة, ومعالجته بسهولة. اذا لم تتم معالجته, في الغالب يؤدي الى مشاكل جهازية. لاتوجد او توجد بشكل نادر تداعيات فموية نتيجة ارتفاع ضغط الدم ولكن ادوية الضغط تسبب غالبا اثار جانبية. الالتهابات الفموية لها غالبا تداعيات على الامراض الجهازية الحادة والمزمنة. التهاب الانسجة ماحول الاسنان هو احد اكثر الامراض الفموية الالتهابية شيوعا. من المحتمل ان يكون الاشخاص المصابين بالتهاب الانسجة ماحول الاسنان اكثر عرضة للتعرض لخطر الاصابة بالنوبات القلبية والامراض الوعائية. البحوث الحديثة قد بينت ان التأثير الالتهابي لالتهاب الانسجة ماحول الاسنان يساعد في تكوين الخثرة الدموية داخل الاوعية. عدة وسائط التهابية توجد في اللعاب ممكن قياسها. الانزيم اللعابي المعدني المحلل لبروتين المطرس-8 قد تم ربطه مع حدوث ارتفاع الضغط و امراض الشريان التاجي. بلاضافة لذلك, المستويات اللعابية من الانزيم المعدني المحلل لبروتين المطرس-8 ترتفع في المتلازمات التاجية الحادة.

اهداف الدراسة: اقيمت هذه الدراسة لقياس الانزيم اللعابي المعدني المحلل لبروتين المطرس -8 وعلاقته بالحالة الصحية للانسجة ماحول الاسنان وسط مجموعة من مرضى ارتفاع ضغط الدم بالمقارنة مع المجموعة الضابطة.

المواد والطرق: تسعون فردا, تتراوح اعمارهم بين 45-50 كانوا يبحثون عن علاج لالام الصدر. جمع العينات تم في مركز ابن البيطار للعلاجات الجراحية في بغداد, العراق. تم تقسيمهم الى مجموعة دراسية (45 مريض) ممن شخصوا بكونهم مرضى ارتفاع ضغط الدم, ومجموعة ضابطة (45 مريض) بدون ارتفاع ضغط الدم. تم استخدام مقياس الصفيحة الجرثومية وفقا لتصنيف (Silness & Ioe, 1964) اما جس عمق جيب اللثة ومستوى الارتباط اللثوي السريري وفقا لتصنيف (Lindhe et al., 1999). تم جمع اللعاب غير المحفز من جميع المرضى حسب (Navazesh & kumar, 2008) لتحليل الانزيم المعدني المحلل لبروتين المطرس-8.

النتائج: اظهرت النتائج ان متوسط قيمة الصفيحة الجرثومية, مستوى الارتباط اللثوي السريري , وجس عمق جيب اللثة اعلى عند المجموعة الدراسية مقارنة بالمجموعة الضابطة مع عدم وجود فرق معنوي. بلاضافة الى ذلك, كان الارتباط ايجابي قوي وسجلت العلاقة فرق معنوي عالي بين قيمة الصفيحة الجرثومية ومستوى الارتباط اللثوي السريري وسط المجموعتين, البروتين اللعابي (الانزيم المعدني المحلل لبروتين المطرس-8) في المجموعة الدراسية سجل متوسط قيمة اعلى من المجموعة الضابطة مع وجود فرق معنوي بين المجموعتين, وسجل ارتباط ايجابي بين البروتين اللعابي (الانزيم المعدني المحلل لبروتين المطرس-8) و قيمة الصفيحة الجرثومية وسط المجموعة الدراسية مع وجود ارتباط معنوي.

الاستنتاجات: سجلت امراض الانسجة ماحول الاسنان نسبة حدوث عالية بين مرضى ارتفاع ضغط الدم, وجد ايضا ان البروتين اللعابي (الانزيم المعدني المحلل لبروتين المطرس -8) له تأثير على حالة الانسجة ماحول الاسنان في المجموعة الدراسية.