DOI: 10.5937/bjdm2403185K

BALKAN JOURNAL OF DENTAL MEDICINE

ISSN 2335-0245

Evaluation of Salivary MMP Levels in Periodontitis Patients with Uncontrolled Diabetes

Gökhan Kasnak, Emre Cantürk Ural, Liubov Karagülle

Department of Periodontology, Faculty of Dentistry, Istanbul Kent University, Istanbul, Turkey

SUMMARY

Background/Aim: *Type 2 diabetes mellitus (T2DM) and periodontitis are both characterized as chronic inflammatory disorders that have a mutually influential association. Dysregulation of glucose levels in individuals with T2DM has a significant impact on the pathophysiology and equilibrium of inflammatory mediators. Multiple mediators are produced throughout the inflammatory cascade of periodontitis and T2DM, including matrix metalloproteinases (MMPs). Objective of this study was to determine if the levels of MMP-3, -8, -9 are attributed to grade III periodontitis patients with uncontrolled T2DM.* ***Material and Methods:*** *Non-smoker 75 individuals with Grade III periodontitis were recruited and were divided into three groups according to the stages of their periodontal status. 75 systemically healthy individuals without periodontitis were served as control. Periodontal clinical metrics, plaque score, gingival bleeding index, probing pocket depth, and clinical attachment level, for each participant were recorded. Blood and whole saliva samples (WSS) were obtained from every participant. Hemoglobin A1c (HbA1c) levels were determined using blood samples, whereas levels of MMP-3, -8, -9 were determined using WSS. Levels of MMPs were assessed using an enzyme-linked immunosorbent assay (ELISA) method.* ***Results:*** *Overall MMP levels and clinical periodontal scores of the Stage III periodontitis patients were significantly higher than healthy individuals ($p<0,01$). Stage III Grade C periodontitis patients had severely increased MMP-3, -8, -9 levels (196,2 ± 2,8; 292,9±1,9; 128,6 ± 3,5 ng/mL, respectively) in comparison to healthy participants (5,2 ± 2,3; 4,3 ± 3,1; 6,1 ± 2,5 ng/mL, respectively) ($p<0,01$). No significant difference was found when Stage III Grade C and B patients were compared regarding the levels of MMP- 3 and -9 ($p>0,05$).* ***Conclusions:*** *Findings of our investigation indicate a potential association between T2DM and increased levels of MMP-3, -8, and -9, which may contribute to the progression of periodontal tissue degradation. In the limitations of our study, MMP-8 exhibited more sensitivity as a marker for periodontal inflammation among the other MMPs that were investigated in this study.*

Keywords: Matrix metalloproteinase, Saliva, Periodontitis, Diabetes

ORIGINAL PAPER (OP)
Balk J Dent Med, 2024;185-189

Introduction

Periodontitis is recognized as a persistent inflammatory illness that leads to the degradation of the periodontal ligament and alveolar bone, which are essential for tooth support. The prevalence rate of this phenomenon is notably high, exerting a detrimental impact on individuals' overall quality of life[1]. According to the findings of a meta-analysis study, diabetes is a significant risk factor for periodontitis. The risk of

developing periodontitis in diabetics is about three times as high as in the general population[2]. Furthermore, while diabetes is recognized to increase the risk for periodontitis, it is established that periodontal inflammation has an impact on glycemic control. This evidence explains why there is a connection between the two conditions[3]. Interfering with the host's immune-inflammatory response has been proposed as a potential mechanism by which hyperglycemia can have a role in the development of periodontitis[4].

Matrix metalloproteinases are a category of zinc-dependent endopeptidase enzymes that are collectively known as matrix metalloproteinases (MMPs). These matrix metalloproteinases are secreted by resident and chemoattracted immune cells as part of the host's response to bacterial assault. These enzymes are accountable for the majority of the extracellular matrix degradation that occurs in tissues, whether they are healthy or pathological[5]. MMP-8 (also known as collagenase-2) and MMP-9 (also known as gelatinase-B) are the MMPs that are most commonly engaged in the degradation of periodontal tissue[6]. Polymorphonuclear leukocytes are responsible for the secretion of the vast majority of the matrix metalloproteinases that may be found in saliva[7]. Because tissue inhibitors of metalloproteinases (TIMPs) work to counteract the action of matrix metalloproteinases (MMPs), an imbalance between the two enzymes can play a role in the advancement of periodontal disease[7,8].

Researchers have begun to recognize how significant the inflammatory pathway actually is in relation to this link. Inflammatory indicators are able to be targeted and analyzed in order to determine the nature of this connection, and MMPs are essential markers, the significance of which has been brought to light in recent research as a potential biomarker. MMP-8 and MMP-9 are the major MMPs that are present in chronic periodontitis. Salivary MMP-8 and crevicular MMP-2, which were recognized to serve as indicators of periodontal disease, have been the subject of research; however, there is a paucity of information on how type-II diabetes mellitus (T2DM) influences the levels of MMP-9 in individuals with periodontitis[9,10].

In 2018, a revised categorization of periodontal diseases was implemented in order to address issues that remained unanswered from the prior classification. Necrotizing periodontal disease, periodontitis as a manifestation of systemic disease, and periodontitis are the three distinct kinds of periodontitis that have been distinguished based on their respective pathophysiologies[11]. In addition, risk factors were not formally included in the periodontal disease classifying system that was implemented in 1999; rather, they were utilized as an identifier to characterize the patient as a smoker or a diabetic mellitus patient. The glycemic level in diabetes and the habit of smoking are both recognized as grade modifiers and risk factors according to the 2018 categorization. It has been hypothesized that this circumstance would make it easier for doctors to make decisions on the prognosis of their patients[12,13].

Extensive research has been conducted to investigate the role of certain collagenases in the etiology of periodontal diseases due to their potential as a reliable biomarker for the identification of periodontitis in oral fluids. However, up to our present knowledge the relationship between the Stage III periodontitis patients and uncontrolled T2DM based on the effect of the Grading (Gr-A, Gr-B, and Gr-C) on the levels of MMPs has not been pointed out. The purpose of this research was to establish whether patients with uncontrolled T2DM have levels of MMP-3, -8, and -9 that are similar to those found in Stage III Gr-A, Gr-B, and Gr-C periodontitis patients.

Material and Methods

This cross-sectional study carried out based on the data of a total of 150 participants. The data was obtained from an earlier study conducted by Kasnak et al**.** Ethics committee approval was obtained from the Istanbul University Faculty of Dentistry (2017/41).

A single calibrated examiner (G.K.) carried out periodontal examinations, including plaque index (PI),[18] gingival index (GI),[19] clinical attachment level (CAL), bleeding on probing (BOP),[20] and probing pocket depth (PPD) measurements at four sites of all teeth by using a periodontal probe (UNC-15, Hu-Friedy, Chicago, IL, USA). Alveolar bone loss of periodontitis patients was observed from orthopantomographs. Orthopantomographs were taken from all individuals using an extra oral imaging system (KODAK 9000 3D, Carestream Dental LLC, Atlanta, GA, USA).

All study participants had classified according to the 2018 periodontal disease classification. 75 patients who had clinical attachment loss ≥ 5mm and teeth loss ≤ 4 was recruited as test group and separated into three groups based on grading, which was performed according to HbA1c levels of the patients as follows: Grade-A: no DM, Grade-B: HbA1c <7%, or Grade-C: HbA1c ≥7%. 75 subjects who had no clinical attachment loss and HbA1c<6% as served control group.

Women who were pregnant or breastfeeding, smoking patients and who had acute or chronic medical conditions, active caries lesions, and patients who had received orthodontic treatment or periodontal therapy at least three months before the data collection were not included in the study.

Saliva Samples Collection

Following the recording of the clinical parameters discussed earlier, the individuals in both groups had their saliva samples obtained to determine the amount of

MMP-3, -8, and -9 present in their bodies. Before taking a sample from the participants, they were given a citrus fruit to smell (either a lemon or an orange). This causes the salivary glands to generate an abnormally large volume of saliva in response to the stimulation. The participants were then given distilled drinking water to gargle with for one minute, and they were instructed to thoroughly clean their mouths. After then, it was requested that the subjects expectorate the water. Following an oral rinse for five minutes, the participants were then instructed to spit into a sterile falcon tube containing 15 milliliters. The participants were advised to let their saliva build at the floor of their mouths and then let it run into the saliva collection tube unintentionally. These samples have been kept at a temperature of -80 °Celsius for further examination.

Before running the ELISA test, the saliva samples were prepared for the assay. All saliva samples were taken out from the -80 °C deep freezers and thawed for 1–2 h. After the samples thawed completely, they were put in the centrifuge at 1000g for 20 min. at 5 °C to remove cell debris. Then, the supernatant was removed and stored in small aliquots of 1.5 mL each in Eppendorf tubes (Eppendorf AG, Hamburg, Germany). According to the manufacturer's instructions saliva samples were diluted to test the MMP-3, -8, and -9 levels (Elabscience, Houston, TX, USA).

ELISA Assay

Standard working solution of different concentrations was added to the first two columns, each concentration of solution being added into two wells side by side and 100 L for each well. Similarly, saliva samples were added to the remaining wells, each sample added into two wells side by side (duplication) and 100 L for each well. The plate was covered with plate sealer and put for incubation at 37 °C for 90 min. After this, liquid from each well was removed, and 100 L of biotinylated detection antibody working solution was immediately added to each well. Plate was again covered with sealer, gently mixed up and put into incubation at 37 °C for 1 h. Then, solution from each well was decanted and 300 L of wash buffer was added to each well. The wells were soaked for 1–2 min and then decanted, patting the plate dry against a clean absorbent paper. This washing step was repeated thrice in total. After the washing, 100 L of HRP conjugate working solution was added to each well. The plate was covered again with sealer and incubated at 37 °C for 30 min. When this time had elapsed, the solution from each well was again decanted and the wash process with wash buffer was repeated five times. Then, 90 L of substrate reagent was added to each well. This step was conducted in a dark environment to protect the plate from light, the substrate reagent being light-sensitive. Plate was covered with a new sealer and wrapped in aluminum foil. Then, it was again incubated at 37 °C for 15 min. The plate was observed in incubation after every 5 min for color changes. After 15 min, when ample color change had occurred in all the wells, 50 L of stop solution was added to each well, ensuring that stop solution was added to each well in the same order as that of substrate reagent addition. After this, the plate was inserted into the Varioskan Flash Multimode Reader (Thermo Fisher Scientific, Waltham, MA, USA) and the optical density (OD) value of each well was determined, keeping the wavelength setting at 450 nm. The blank solution well was the background for each kit, and its OD value was subtracted from the OD value derived for each well to obtain the corrected OD. The protocol was repeated for all three MMPs; MMP-3, -8, and -9.

Statistical Analysis

Statistical analysis of the mean difference between the groups regarding MMP levels was conducted using a Kruskal-Wallis one-way ANOVA with the Mann-Whitney U test, while independent was used for the assessment of HbA1c and periodontal clinical metrics. $p<0,05$ was accepted as statistically significant. All data were analyzed using SPSS version 24.0 statistical software (IBM Corp., NY, USA).

Results

All clinical parameters were higher in the Stage III Gr A ($p<0.01$), Stage III Gr B ($p<0.01$), and Stage III Gr C groups compared to periodontally healthy controls (Table 1). There was no substantial difference in age or gender between groups ($p>0.05$).

Table 1. Clinical parameters of the study population. The clinical parameters of the study population were significantly higher than healthy subjects ($p<0.01$)

Clinical Parameters (Mean±Std)	Healty Individuals (n=15)	Grade III Stage A (n=15)	Grade III Stage B (n=15)	Grade III Stage C (n=15)	p value		
PI (%)	9.12±9.30	33.78±22.63	30.73±26.50	31.12±33.46	<0.01	<0.01	<0.01
GI (%)	3.33±12.90	78.45±8.93	79.32±12.96	80.21±10.18	<0.01	<0.01	<0.01
PD (mm)	2.13±0.12	5.39±0.60	5.35±0.31	5.15±0.22	<0.01	<0.01	<0.01
CAL (mm)	0.18±0.78	5.86±0.58	5.66±0.32	5.21±0.43	<0.01	<0.01	<0.01

Overall MMP levels and clinical periodontal scores of the Stage III periodontitis patients were significantly higher than healthy individuals ($p<0.01$). Stage III Grade C periodontitis patients had severely increased MMP-3, -8, -9 levels (196.2 ± 2.8; 292.9±1,9; 128.6 ± 3.5 ng/mL, respectively) in comparison to healthy participants (5.2 ± 2.3; 4.3 ± 3.1; 6.1 ± 2.5 ng/mL, respectively) ($p<0.01$). Likewise, MMP-3, -8, -9 levels of the Stage III Grade C patients were higher than the Stage III Grade A patients (76 ± 4.3; 98 ± 1.6; 80 ± 3.4 ng/mL, respectively) ($p<0.001$). No significant difference was found when Stage III Grade C and B patients were compared regarding the levels of MMP- 3 and -9 ($p>0.05$). However, the MMP-8 levels of patients in Stage III Grade C were found to be significantly higher compared to those in Stage III Grade B (186 ± 2.4 ng/mL) ($p<0.05$) (Table 2).

Table 2. The comparison of MMP levels between the study population and control group

MMPs (Mean±Std)	Healty Individuals (n=15)	Grade III Stage A (n=15)	Grade III Stage B (n=15)	Grade III Stage C (n=15)	p value		
MMP-3 (ng/ml)	5.2±2.3	76.4±4.3	180.5±3.4	196.2±2.8	<0.01	<0.01	<0.01
MMP-8 (ng/ml)	4.3±3.1	98.2±1.6	186.2±2.4	292.9±1.9	<0.01	<0.01	<0.01
MMP-9 (ng/ml)	6.1±2.5	80.1±3.4	120.3±2.1	128.6±3.5	<0.01	<0.01	<0.01

Discussion

Periodontal disease is a persistent infectious condition marked by the deterioration of supportive tissues around the teeth, with a primary focus on the degradation of the extracellular matrix, notably collagen. The MMP (matrix metalloproteinase) family plays a pivotal role in this degradation process. Among them, MMP-2 and -8 are extensively distributed within oral tissues. Particularly noteworthy is the influence of MMP-8, expressed by neutrophils, on the progression of inflammation and its impact on various inflammatory cells in the oral environment[14].

Previous studies examining gingival crevicular fluid have reported heightened expression of MMP-2 and -8 in periodontal disease, indicating their potential diagnostic utility [28]. The mechanism behind this increased expression in periodontal disease is thought to involve Porphyromonas gingivalis and IL-1[15]. To facilitate analysis, the values were subjected to log transformation, as detailed in a prior study[16]. In this study, we explored salivary concentrations of MMPs as potential inflammatory biomarkers for periodontal disease, periodontal status, and HBA1c levels to determine the level of diabetes mellitus which is a modifier of the periodontal disease.

Damage to periodontal tissue resulting from uncontrolled glycemia is attributed to the thickening of the capillary basement membrane. A morphological hallmark of diabetic microvascular complications is the appearance of microaneurysms[17]. During the clinical periodontal evaluation in the current study, individuals with Type 2 Diabetes Mellitus (T2DM) and inadequate glycemic control demonstrated a notable increase in sites displaying gingival redness, clinical attachment loss, and elevated probing depths which are in line with the aforementioned histopathological changes.

Our findings reveal an approximately twofold elevation in MMP levels among individuals with T2DM and Stage III Grade B, and Grade C periodontitis, compared to the control group. This suggests that T2DM induces a heightened release of MMPs during inflammation nearly double that observed in inflammation caused by severe periodontitis alone. Consequently, it can be inferred that these elevated MMP levels in T2DM play a significant role in the periodontal destruction observed in diabetic individuals. Addressing and controlling these elevated MMP levels may contribute to a reduction in the severity of periodontitis in diabetic subjects. In line with our findings, research conducted by Han Na Kim, and Arshad et al. has demonstrated similar outcomes. Han Na Kim et al. reported a reduction in salivary MMP levels following effective periodontal therapy, suggesting a positive response to the intervention[18]. Meanwhile, Arshad et al. identified an increased concentration of MMP-9 in the salivary samples of diabetic patients with periodontitis, aligning with our observations[1]. These parallel results from distinct studies contribute to the growing body of evidence supporting the significance of MMPs in periodontal health and disease, underscoring the consistency of these associations across different research contexts.

This study is subject to certain limitations, primarily stemming from time constraints and limited research grant funding. To enhance the robustness of future investigations, overcoming these limitations would be imperative. Specifically, a larger sample size would afford a more comprehensive exploration of the current hypothesis and results, thereby contributing to a more nuanced understanding of the subject matter.

Conclusions

Clinical changes in poorly managed Type 2 Diabetes Mellitus individuals included reduced gingival redness, increased clinical attachment loss, bleeding, and elevated

MMP-3, -8, and -9 levels, potentially contributing to periodontal degradation. Limitations include MMP-8's higher sensitivity as a marker for periodontal inflammation compared to other MMPs in this study.

References

1. Arshad R, Ismail WA, Zara B, Naseer R, Minhas S, Ansari M, et al. (2022). "Salivary MMP-9 levels in chronic periodontitis patients with type-II diabetes mellitus". *Molecules*. **27** (7): 2174. doi: 10.3390/molecules27072174. PMID: 35408573.
2. Chávarry NG, Vettore MV, Sansone C, Sheiham A (2009). "The relationship between diabetes mellitus and destructive periodontal disease: a meta-analysis". *Oral Health Prev Dent*. **7** (2): 107-127. PMID: 19583037.
3. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. (2012). "Periodontitis and diabetes: a two-way relationship". *Diabetologia.* **55** (1): 21-31. doi: 10.1007/s00125-011-2342-y. PMID: 22057194.
4. Hanes PJ, Krishna R (2010). "Characteristics of inflammation common to both diabetes and periodontitis: are predictive diagnosis and targeted preventive measures possible?" *EPMA J.* **1** (1): 101-116. doi: 10.1007/s13167-010-0016-3. PMID: 23199045.
5. Sorsa T, Tjäderhane L, Salo T (2004). „Matrix metalloproteinases (MMPs) in oral diseases". *Oral Dis.* **10** (6): 311-318. doi: 10.1111/j.1601-0825.2004.01038.x. PMID: 15533204.
6. Gursoy UK, Könönen E, Huumonen S, Tervahartiala T, Pussinen PJ, Suominen AL, et al. (2013). "Salivary type I collagen degradation end-products and related matrix metalloproteinases in periodontitis". *J Clin Periodontol*. **40** (1): 18-25. doi: 10.1111/jcpe.12020. PMID: 23078613.
7. Uitto VJ, Suomalainen K, Sorsa T (1990). „Salivary collagenase. Origin, characteristics and relationship to periodontal health". *J Periodontal Res*. **25** (3): 135-142. doi: 10.1111/j.1600-0765.1990.tb01035.x. PMID: 2163444.
8. Birkedal-Hansen H (1993). "Role of matrix metalloproteinases in human periodontal diseases". *J Periodontol.* **64** (5): 474-484. doi: 10.1902/jop.1993.64.5s.474. PMID: 8315570.
9. Collin HL, Sorsa T, Meurman JH, Niskanen L, Salo T, Rönkä H, et al. (2000). „Salivary matrix metalloproteinase (MMP-8) levels and gelatinase (MMP-9) activities in patients with type 2 diabetes mellitus". *J Periodontal Res*. **35** (5): 259-265. doi: 10.1034/j.1600-0765.2000.035005259.x. PMID: 11005153.
10. Basiroh Abdullah NF, Faisal GG, Radeef AS, Suhaila MA (2017). "Assessment of llness perception of diabetic patients with periodontitis". *J Int Dent Med Res*. **10** (1): 100-107.
11. Tonetti MS, Greenwell H, Kornman KS (2018). "Staging and grading of periodontitis: Framework and proposal of a new classification and case definition". *J Periodonto*l. **89** (1): S159-S172. doi: 10.1002/JPER.18-0006.
12. Beşiroğlu E, Yüksek SK (2023). „Comparison of 1999 and 2018 Periodontal Disease Classifications in Diabetic and Smoker Groups: A Retrospective Study". *Turkiye Klinikleri. Dishekimligi Bilimleri Dergisi.* **29** (1): 121-130. doi: 10.5336/dentalsci.2022-89922
13. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. (2018). "Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions". *J Clin Periodontol*. **45** (20): S162-S170. doi: 10.1111/jcpe.12946. PMID: 29926490.
14. Kang MG, Cha HJ, Song SH, Park JW, Suh JY, Lee JM (2005). "MMP-2, MMP-8 Expression in gingival tissue of Chronic Periodontitis associated to Type 2 Diabetes Mellitus". *J Korean Acad Periodontol.* **35** (3): 661-674. 10.5051/jkape.2005.35.3.661.
15. Kuula H, Salo T, Pirilä E, Tuomainen AM, Jauhiainen M, Uitto VJ, et al. (2009). „Local and systemic responses in matrix metalloproteinase 8-deficient mice during Porphyromonas gingivalis-induced periodontitis". *Infect Immun*. **77** (2): 850-859. doi: 10.1128/IAI.00873-08. PMID: 19029300.
16. Han DH, Shin HS, Paek D, Kim HD (2012). "Gingival crevicular fluid levels of matrix metalloproteinases cross-sectionally related to periodontitis and metabolic syndrome in community Koreans". *J Clin Periodontol.* **39** (12): 1125-1131. doi: 10.1111/jcpe.12014.: 23036163.
17. Nitta H, Katagiri S, Nagasawa T, Izumi Y, Ishikawa I, Izumiyama H, et al. (2017). "The number of microvascular complications is associated with an increased risk for severity of periodontitis in type 2 diabetes patients: Results of a multicenter hospital-based cross-sectional study". *J Diabetes Investig*. **8** (5): 677-686. doi: 10.1111/jdi.12633. PMID: 28129466.
18. Kim HN (2022). "Changes in salivary matrix metalloproteinase-3,-8, and-9 concentrations after 6 weeks of non-surgical periodontal therapy". *BMC Oral Health*. **22** (1): 175. doi.org/10.1186/s12903-022-02185-3.

Received on July 2, 2024.
Revised on September 19, 2024.
Accepted September 27, 2024.

Conflict of Interests: Nothing to declare.
Financial Disclosure Statement: Nothing to declare.
Human Right Statement: All the procedures on humans were conducted in accordance with the Helsinki Declaration of 1975, as revised 2000. Consent was obtained from the patient/s and approved for the current study by national ethical committee.
Animal Rights Statement: None required.

Correspondence

Gökhan Kasnak

Department of Periodontology, Faculty of Dentistry
Istanbul Kent University, Istanbul, Turkey
e-mail: gkasnak@gmail.com / gokhan.kasnak@kent.edu.tr