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Expression of Salivary Matrix Metalloproteinase-3 in Periodontitis Patients

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ABSTRACT

Background: Periodontitis is a chronic inflammatory disease characterized by the destruction of supporting oral tissues. The condition involves unique host responses within the oral environment, including the production of selected matrix metalloproteinases (MMPs) and certain inflammatory mediators that take part in tissue remodeling. The study aimed to evaluate MMP-3 expression levels in saliva samples collected from healthy and diseased subjects.

Methods: 82 study participants were enrolled in this case-control study through a convenience sampling technique from the Periodontology OPD of the Ziauddin College of Dentistry, Karachi, Pakistan, from January 2023 to January 2024. Participants were divided into cases and controls depending on their clinical periodontal status. Saliva samples were collected from all participants using the Passive Drool Method. Analysis of collected samples was performed through ELISA Assay Method to determine quantitative expression levels of MMP-3. SPSS software version 24 was used to run the T-test and the Mann-Whitney Test for two-group comparisons. Correlation analysis of MMP-3 protein expression levels with periodontal parameters was performed by Pearson's Correlation. ROC curve was produced to determine the best cut-off value using Youden's Index with 0.05 level of significance.

Results: Salivary expression levels of MMP-3 was significantly higher in periodontitis patients when compared to controls. MMP-3 expression was positively correlated with periodontal parameters including periodontal pocket depth, clinical attachment loss, bleeding on probing, and plaque score.

Conclusion: MMP-3 was strongly associated with periodontitis, and its expression levels could be implicated in determining disease progression.

Keywords: Matrix Metalloproteinase, Periodontitis, Extracellular Matrix, Saliva.

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INTRODUCTION

Periodontitis is a chronic inflammatory condition that causes destruction of the tooth's supporting structures. This includes the gingiva, root cementum, periodontal ligament, and surrounding alveolar bone[1]. The disorder results in the loss of periodontal tissues due to a combination of interactions taking place between bacteria and the body's immune response. This inflammation is brought on by a biofilm of plaque which surrounds the tooth surface and ultimately results in periodontal attachment loss, while tooth loss may take place in severe cases[2].

The main etiology of the disease is considered to be anaerobic bacteria but the progression of the condition arises due to host response. This is shown to be influenced by a series of factors including both environmental as well as genetic variations[3]. Periodontal disease development begins with the interactions of several molecular pathways comprising mediators involved in inflammation. Common examples include matrix metalloproteinase (MMPs), growth factors, pro-inflammatory cytokines, and the appropriate regulators and inhibitors of these molecules[4].

MMPs are defined as a specific class of endopeptidases that are dependent on zinc and calcium for their proteolytic activity. They are released by various cells including endothelial cells, fibroblasts, macrophages, and osteoblasts. Their activity is intricately regulated by a class of tissue inhibitors of metalloproteinase. Furthermore, they have been proven to play a pivotal role in the breakdown of important structural components that are found in the extracellular matrix. This predominantly includes degradation of proteins such as laminin, collagen, elastin, proteoglycan, fibronectin, and integrin. Concurrently, scientific evidence proves MMPs are involved in the destruction of the collagen matrix that arises during degradation of the periodontal ligament and alveolar bone in periodontal disease. At the same time, they have been proven to impact resorption of bone by activating osteoclasts and allowing for their differentiation while also enabling direct destruction of the bone's collagen matrix[5].

MMPs comprise various subtypes, depending on their specific substrates and distribution inside tissues. MMP-3 is also known as stromelysin-1 and has been highlighted to be a leading proteolytic enzyme when compared to other notable members from the MMP family. This has to do with its influential role in activating the latent MMP subtypes like MMP-7,8,9, and 13. Similarly, evidence claims that MMP-3 also has a crucial role in starting the process of collagen breakdown in patients with periodontitis[6].

Many studies have spoken about the alterations in inflammatory biomarkers present in saliva and GCF for periodontal patients. This includes their use for diagnosis purposes as well as treatment planning. Since MMP-3 is known to be an integral player in activating the latent cascade for several other MMPs, its significance in playing a part during the breakdown of the periodontal matrix requires further investigation[7].

While several studies have been documented which elucidate the role of MMPs in periodontitis, those specifically targeting MMP-3 and involving the Pakistani population are limited. Furthermore, studies carried out in other areas around the world with different ethnicities produced inconsistent results. For this reason, greater research in this domain is recommended to evaluate the diagnostic potential of MMP-3 and its use for targeted therapy purposes in periodontal disease.

The present research aimed to study the expression levels of salivary MMP-3 in participants with and without periodontitis. It was hypothesized that in a subset of the Pakistani population diagnosed with periodontitis, there will be a change in salivary MMP-3 expression levels when compared to controls, and therefore an association between MMP-3 and periodontal disease exists.

METHODS

In this case-control study, 82 patients from the Department of Periodontics, Ziauddin College of Dentistry, Karachi, Pakistan, ethical approval reference code 5820822HAOM, were enrolled between January 2023 and 2024. Non-probability convenience sampling technique was used and the sample size was calculated using OpenEpi Version 3, Open-Source Calculator. Study subjects were divided into cases and controls depending on their clinical periodontal condition. Cases included individuals aged 20 to 60 years belonging to both genders with generalized moderate or severe periodontitis as per the American Association of Periodontology (AAP) 2017 Classification of Periodontal Diseases and Conditions. Participants with generalized periodontitis in ≥30% of all teeth, detectable buccal clinical attachment loss (CAL) of ≥3 mm, detectable interdental CAL in ≥2 non-adjacent teeth, and periodontal pocket depth (PPD) ≥ 4mm in ≥ 2 teeth non-adjacent teeth were included. Controls included individuals aged 20 to 60 years belonging to both genders with a healthy periodontium with no signs of gingival inflammation. This included bleeding on probing (BOP) scores being less than 10%, periodontal pocket depth (PPD) ≤ 3mm, and no clinical attachment loss (CAL) [8]. Patients with infections/inflammatory conditions, systemic diseases, smoking habits, pregnancy/lactation, or history of undergoing periodontal treat-

ment in the past six months were excluded from the study. Similarly, those taking anti-inflammatory drugs, antibiotics, dietary supplements, or immunosuppressant medications were also excluded.
The Unstimulated Whole Saliva (UWS) was collected from all participants using the Passive Drool Method during the early hours of the morning (0900 to 1200). From each patient, approximately 2 to 5ml of unstimulated whole saliva was collected in sterilized falcon tubes. Following the collection, the saliva samples were immediately placed inside an ice-filled container with an approximate temperature of -20 degrees centigrade. Micropipettes were used to transfer aliquots of every sample into Eppendorf tubes for centrifugation before being stored at -20 degrees centigrade inside the lab. Analysis of the collected samples through enzyme-linked immunosorbent assay (ELISA) was conducted within 6 months to determine salivary MMP-3 levels.
ELISA was performed to check the quantitative expression levels of MMP-3 protein following kit manufacturers' protocols. Colorimetric readings were obtained using a 96-well plate ELISA reader MultiSkan Sky spectrophotometer at specific wavelength. In the end, the microplate reader was run and measurements were conducted immediately at 450nm.

SPSS software version 24 was used to carry out statistical analysis. Mean and Standard Deviation were used to express the continuous variable study results while frequency and percentages were used for categorical variables. To determine normality of the data, Shapiro Wilk's Test was used. A T-test was used for 2 group comparisons in case of parametric data while the Mann-Whitney Test was used in case of non-parametric data. Correlation analysis of the MMP3 protein expression levels with periodontitis parameters was performed by Pearson's Correlation as per normality check of the variables. A p-value of ≤0.05 was taken as statistically significant. A ROC curve was produced to tell different coordinate points and the best cut-off value was then selected using Youden's Index.

RESULTS

Table 1. Showing Demographics and Periodontal Parameters of Study Subjects

Variables	Min	Max	Mean ±SD	Median (IQR)
Age	19	59	32 ± 10.5	30 (17)
BMI	12	35	22 ± 4.4	22.4 (7)
GI	0	3	1.47 ± 1.3	2.0 (3)
MI	0	3	1.00 ± 1.09	1.0 (2)
PI	0	3	1.53 ± 1.28	2.0 (3)
BOP	0	1	0.58 ± 0.49	1.0 (1)
PPD	1.0	5.5	2.96 ± 1.48	3.5 (2.5)
CAL	0	7.0	2.93 ± 2.97	4.5 (5.8)
MMP3	2.05	8.45	4.86 ± 1.53	4.4 (2.5)

The study results of 41 cases with periodontitis and 41 control subjects showed that Mean and standard deviation values, which were calculated for all the numeric variables in both study groups are presented in **Table 1**. Of the 82 patients, demographic data distributions were determined for gender, age range, and body mass index (BMI) scores. The study proved how the mean demographic variables including age and gender were not significantly different among each group ($p > 0.05$). On the other hand, the periodontal parameters including PPD, CAL, BOP, and Plaque Score were proven to be statistically greater for the Case Groups compared to the Controls (all $p < 0.001$).

Table 2. Showing Demographics and Oral Hygiene Practices

Variables	Frequency (%)
Gender	
Male	38 (46.3%)
Female	44 (53.7%)
Smokeless Tobacco	
Yes	31 (37.8%)
No	51 (62.2%)
Brushing Habits	
None	15 (18.3%)
Once Daily	19 (23.2%)
Twice Daily	42 (51.2%)
Thrice Daily	6 (7.3%)

For all categorical variables, frequency and percentage were calculated and are presented in **Table 2.**

Mean and standard deviation values were calculated for the quantification of salivary MMP-3 levels. As a whole, the mean MMP-3 levels detected in saliva of the case group was significantly higher than that observed in the control group. ($p < 0.001$).

Table 3. Comparing MMP3 Levels Between Cases and Controls

Groups	N	Mean Rank	p-Value
Cases	41	60.21	<0.001
Controls	41	22.79	<0.001

Salivary MMP3 levels for both periodontitis cases and control subjects were calculated and are shown in **Table 3**. The salivary levels of MMP3 in the periodontitis case group were much higher than the control group. Using the data calculated, an ROC curve was produced and shown as **Figure 1**. This was designed to determine the accuracy and also differentiate between periodontitis cases and controls by plotting sensitivity against specificity at various cut-off points. This optimal cut-off value was selected with Youden's Index Method. The best cut-off point was found to be 4.6. Hence, it can be claimed that if MMP-3 levels are 4.6 or above, Periodontitis will occur. The area under the curve (AUC) symbolizes the test's discriminatory power.

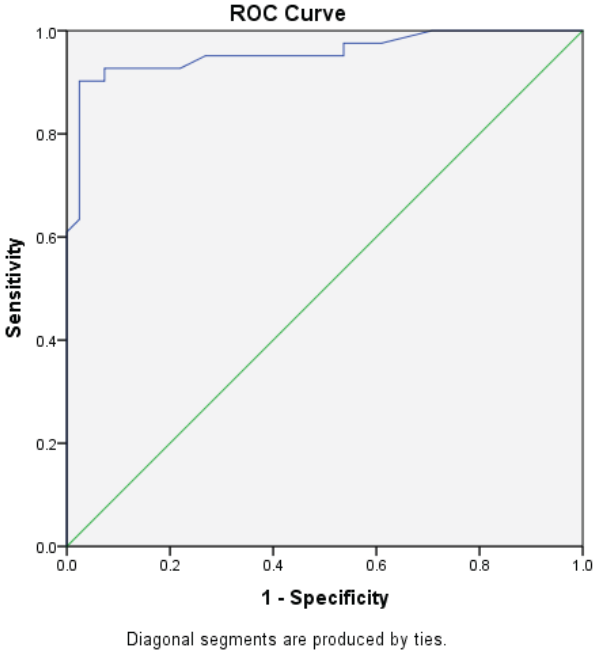

Figure 1. Showing Receiver Operating Curve (ROC)

DISCUSSION

Periodontal disease is known to involve inflammation of the tooth's supporting structures[11]. It results when there is a disturbed equilibrium between the oral biofilm and the host's response[12]. This is why a wide range of genetic and environmental factors have been proven to contribute to the disease[13]. The process begins with anaerobic bacteria that initiate the body's immune response through a complex framework featuring a range of molecular pathways. The main target for degradation is the extracellular matrix, collagen in particular, where the MMP family has been proven to play a major role in this process[14].

Previous studies have shown how MMP-3 values in the GCF tend to be higher in periodontitis sites as compared to healthy areas. This has to do with the fact that bacterial stimuli end up initiating MMP3 expression[15]. MMP-3 is an enzyme needed to destroy extracellular matrix proteins including laminin. At the same time, it is required to start the cascade activation for a range of other MMPs such as MMP-1, 8, and 9[10,16].

The great significance of the MMP-3 gene cannot

be denied. The MMP-3 gene polymorphism was proven to impact an individual's susceptibility to periodontitis. This paved the way to how genetics can serve as important modulators for periodontal tissue destruction[17].

This current study's findings showed how subjects with periodontitis had increased mean salivary MMP-3 protein expression levels when compared to healthy controls. Subjects with periodontitis had increased mean salivary MMP-3 protein expression levels when compared to healthy controls. The results are in exact accordance with the results published in another study which also proved levels of MMPs significantly decrease after surgical periodontal therapy[9]. The findings are also aligned with other studies, which concluded that MMP-3 and not MMP-9 was implicated in the manifestation of chronic periodontitis and how their levels in GCF markedly rose in periodontitis patients[18, 19].

Literature has similar spoken about the innovative role of certain drugs serving as MMP inhibitors as an alternative and useful strategy for periodontitis[20]. Several substances and natural products might restore periodontal tissue health by inhibiting MMPs and similar molecular cascades[21]. While serving an adjunctive role to routine scaling and root planning techniques[22].

MMPs cause uncontrolled breakdown of the periodontal tissues which is considered as the most cardinal sign of periodontitis[23]. While periodontal inflammation and polymicrobial biofilm are important to understanding the pathology of this condition, it's also crucial to understand the inflammatory response on a molecular level [24]. Due to limitations such as sample size, the findings of this study should be interpreted with caution as it necessitates the domain to be explored further to better validate the outcome during future investigations. Nevertheless, the results obtained could be of great assistance in regulating periodontal health and coming up with the best treatment strategy to better combat this alarming oral health concern[25].

CONCLUSION

This research study was able to demonstrate a significant increase in the salivary expression as well as the activity of MMP-3 in patients with Periodontitis. Therefore, detecting its levels in saliva could give rise to a modern alternative for monitoring the activity of enzymes such as MMPs.

LIST OF ABBREVIATIONS

MMP-3- Matrix Metalloproteinase
CAL- Clinical Attachment Loss
BOP- Bleeding on Probing
PPD- Periodontal Pocket Depth
ROC- Receiver Operating Characteristic Curve
AUC- Area Under Curve
UWS- Unstimulated Whole Saliva
GCF- Gingival Crevicular Fluid
ELISA- Enzyme-Linked Immunosorbent Assay
AAP- American Association of Periodontology
BMI- Body Mass Index

DECLARATIONS:

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CONFLICT OF INTEREST

All the authors hereby declare that there is no conflict of interest.

FUNDING

N/A

PATIENT CONSENT

Verbal and written informed consent were obtained from all patients.

ETHICAL APPROVAL

The study was approved by the Ziauddin University Ethics Revie Committee Reference Code: 5810822UGMED.

AUTHORS' CONTRIBUTIONS

SHA: Data collection, formal analysis, investigation, methodology, writing, editing, funding, **SSQ:** Investigation, supervision, editing, review. **RI:** Conceptualization, investigation, project administration, supervision, validation, review, and editing. **FS:** Review and editing. All the authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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