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Original Article

Matrix metalloproteinase 9 level changes in gingival crevicular fluid samples of teeth with acute and chronic apical periodontitis

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ABSTRACT

Background: This study investigates the influence of dental pulp and periapical status on inflammatory mediators, particularly matrix metalloproteinases (MMPs), which contribute to tissue destruction around the teeth and the development of periodontitis. This study aimed to compare MMP-9 levels in the gingival crevicular fluid (GCF) between the cases of acute apical periodontitis, chronic apical periodontitis, and healthy controls before and after root canal treatment (RCT).

Materials and Methods: This prospective, cohort study involved 19 samples each of acute and chronic periodontitis cases, both before and after RCT, along with 18 samples from healthy control teeth. The samples were collected from the GCF using paper cones. After 2 weeks of RCT, the process was repeated. MMP-9 levels were measured using the enzyme-linked immunosorbent assay technique. Statistical analysis was performed using the paired *t*-test and analysis of variance test and the significance level was set at < 0.05.

Results: Before treatment, there was no significant difference in MMP-9 levels between the healthy (0.476 μg/μL) and acute (0.48 μg/μL) groups; however, significant differences were observed between the healthy and chronic (0.534 μg/μL) groups. In addition, MMP-9 levels differed significantly between the acute and chronic groups before treatment. Post-treatment, the healthy group showed no notable difference compared to either patient group. However, a significant difference was observed between the acute (0.445 μg/μL) and chronic (0.491 μg/μL) groups after treatment.

Conclusion: Our findings suggest that MMP-9 levels in GCF increase during periapical inflammation and decrease after endodontic treatment. MMP-9 may serve as a potential diagnostic biomarker for pulp and periapical inflammation, enhancing our understanding of these clinical conditions and informing future therapeutic strategies.

Key Words: Gingival crevicular fluid, matrix metalloproteinase-9, periapical periodontitis

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INTRODUCTION

Apical periodontitis is a common inflammatory disease that leads to tooth loss along with caries. Apical periodontitis represents a local immune response against invading microorganisms from an infected root canal space to the periapical region.[1]

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Despite advances in imaging, diagnosing pulp and periapical issues remains difficult, therefore exploring molecular diagnostics may improve understanding and enable the development of chairside tests. However, limitations persist due to the inability of current dental imaging methods to assess soft-tissue inflammation or bacterial infiltration in the dental pulp.[2]

Host cells release various inflammatory mediators, proinflammatory cytokines, and growth factors as an immune response during periapical infection.[3] Matrix metalloproteinases (MMPs) are vital endopeptidases that exhibit significant importance.[4] The presence of pathogens stimulates host cells to increase the release of MMPs, which are considered indirect mechanisms in tissue destruction during periodontitis. The healthy periodontal ligament is protected from the proteolytic attack of MMPs by Tissue Inhibitors of Metalloproteinases (TIMPs). In chronic periodontitis, the level of TIMP is low and insufficient to inhibit MMPs. As a result, progelatinase (pro-MMP-9) is activated and it leads to the accumulation and activation of inflammatory cells such as lymphocytes and neutrophils. Consequently, changes in inflammatory mediators and secretion of inflammatory proteases will occur.[5]

MMP-9 or gelatinase B is reported to be a multidomain enzyme that functions in both acute and chronic inflammatory diseases.[6] MMP-9 is a gelatinase that is mainly secreted by neutrophils, and during the active and progressive phase of periodontitis, the level of MMP-9 in the periodontal crevicular fluid increases significantly.[7] MMP-9, by breaking down type IV collagen, disrupts basement membranes associated with tumor infiltration and metastasis.[8] It is also necessary to initiate clastic bone resorption by removing collagen from the bone structure before demineralization begins. MMP-9 plays a crucial role in periodontal bone destruction by degrading extracellular matrix components and promoting inflammatory responses, which accelerates tissue breakdown and bone loss in periodontal disease.[7,9]

In addition, it has been suggested that root canal treatment (RCT) can interfere with the expression of MMPs.[10] Therefore, MMP-9 can be used as a diagnostic biomarker for the accurate and timely diagnosis of the periapical condition.[2] This study aims to compare the level of MMP-9 in gingival crevicular fluid (GCF) in the conditions of acute and chronic apical periodontitis before and after RCT in comparison with the healthy periapical tissue. The null hypothesis was that there is a difference in MMP-9 levels in the GCF between cases of acute and chronic periodontitis, between periodontitis and healthy cases, and before and after RCT.

MATERIALS AND METHODS

The study population

In this prospective, cohort study, the samples were obtained from 38 patients from 38 teeth referred to the Islamic Azad University of Medical Sciences, School of Dentistry, Endodontics Department in 2022–2023 who have been diagnosed with acute periapical periodontitis and chronic periapical periodontitis (19 cases of chronic apical periodontitis and 19 cases of acute apical periodontitis). The sample size was determined using the Bonferroni formula as follows and based on the information obtained from a study[10] on MMP levels in the GCF in the target groups, with $n1 = n2 = n3 = 15$. The samples were taken before and after RCT. The control samples were taken from the opposite healthy tooth of the patients. These control samples were obtained from 18 intact and unrestored teeth without caries. The study was performed after obtaining informed consent from the patients and was approved by the Research Ethics Committee of Tehran Islamic Azad University of Medical Sciences (Thesis code: 25155). Furthermore, the Helsinki Declaration was read and the guidelines were followed. Groups were matched regarding age and gender. The exclusion criteria were the presence of inflammatory diseases of nondental origin, the presence of ulcers or oral inflammation, systemic diseases, long-term use of anti-inflammatory drugs, and smoking. Participation in the study was entirely voluntary and all patients, regardless of their participation in the study, received appropriate treatment for their condition.

Gingival crevicular fluid sampling

The area was isolated by a cotton roll and dried with a gentle flow of air. For sampling, four paper cones were placed in the sulcus of the tooth for 30 s in the mesial, distal, buccal, and lingual surfaces. After 2 weeks from their RCT, this procedure was repeated. If the samples were contaminated with blood and saliva, sampling was repeated. These actions were also performed in the control teeth. The samples were transferred to a sample tube containing 100 µl of Tris-HCl buffer, pH 7.5, with 0.15 M NaCl and

1 mM CaCl2, and were placed on the rotator for 3 h and then kept at − 20°C. The amount of MMP-9 was calculated in μg/μL by the enzyme-linked immunosorbent assay (ELISA) with a Human MMP-9 Elisa Kit (Sigma Aldrich Inc., Missouri, US).

Patients' subjective symptoms and clinical examination

Patients diagnosed with apical acute periodontitis reported experiencing pain primarily during percussion. However, they also felt pain during chewing, biting, and sometimes spontaneously and may or may not respond to pulp viability tests. The radiographic image of the tooth generally showed minimal widening of the periodontal ligament. The teeth in cases of chronic apical periodontitis had no clinical symptoms, these teeth did not respond to pulp vitality tests, and radiography images showed apical radiolucency. These teeth were generally not sensitive to chewing pressure, but the patient had a different feeling to the percussion test.

Root canal treatment procedure

After preparing the access cavity, apical patency was confirmed, and a #15 K-file (Dentsply-Maillefer, Switzerland) was used to establish the 1 mm shorter than apex working length. A single operator prepared all root canals. Bio-RaCe (FKG, La-Chaux De Fonds, Switzerland) was used for canal preparation in all teeth, following the crown-down technique as per the manufacturer's instructions. Files were discarded after being used in five canals. Each canal was irrigated with 2 mL of 2.5% sodium hypochlorite (Cerkamed, Poland) using a 27gauge needle after using each file. A final irrigation with 5 mL of distilled water (Farazdental, Iran) was performed. The root canals were then dried with paper points (Gapadent, Tianjin, China) and filled with gutta-percha (Gapadent, Tianjin, China) and AH Plus sealer (Dentsply-Maillefer, Switzerland) using the lateral compaction technique.

Statistical analysis

The statistical analysis was performed using the SPSS software v. 22 (SPSS Inc., Chicago, IL, USA) with a significance level of $P < 0.05$. Descriptive statistics indicated the data distribution. Paired *t*-test and analysis of variance were used to compare the data distribution between the study groups. Pearson's correlation analysis was performed to calculate the correlation between study groups, gender, and age using Python 3.8.10.

RESULTS

The statistical analysis indicates that MMP-9 values before and after RCT in the study groups had a normal distribution. Pairwise comparisons for MMP-9 in the studied groups showed no notable difference between the MMP-9 values before treatment of the acute group (0.48 μg/μL) and the normal group (0.476 μg/μL) ($P = 1$) [Table 1]. However, there was a significant difference between the normal and chronic (0.534 μg/μL) groups before treatment ($P = 0.0001$). A noticeable difference was found between acute and chronic groups both before and after the RCT ($P = 0.001$). The MMP-9 levels after the treatment for the acute and chronic group were 0.445 and 0.491 μg/μL, respectively. There was no significant difference between the normal group values and any of the two groups after the treatment ($P = 0.009$).

The results of the paired *t*-test indicate that there were remarkable differences between the conditions before treatment and after treatment. In the acute group, the average MMP-9 after RCT is 0.035 μg/μL less than before treatment, while for the chronic group, this difference was 0.043 μg/μL [Figure 1].

For chronic conditions, the correlation between gender and MMP-9 levels before and after treatment was − 0.076 and 0.048, respectively. These results suggest weak correlations. For acute conditions, the correlation between gender and MMP-9 levels before and after treatment was − 0.206 (moderately negative) and 0.202 (moderately positive), respectively [Figure 2]. For the healthy group, the correlation is moderately positive (0.207), indicating a moderate positive, but not strong relationship between gender and MMP-9 levels in healthy individuals.

Furthermore, regarding the correlation between age and MMP-9 levels, for chronic conditions, before and

Table 1: Levels of matrix metalloproteinases - 9 (μg/μL) before and after root canal treatment in three study groups

Group	MMP-9 level	
	Before RCT, mean±SD	After RCT, mean±SD
Periapical chronic periodontitis	0.534±0.037	0.491±0.047
Periapical acute periodontitis	0.480±0.04	0.445±0.043
Healthy teeth	0.476±0.046	

MMP-9: Matrix metalloproteinases-9; RCT: Root canal treatment; SD: Standard deviation

after treatment, it was 0.104 and 0.159, respectively. For the acute group, it was −0.027 and −0.256 before and after RCT, respectively. The strongest correlation is in the healthy group (0.39), suggesting as age rises, the MMP-9 level increases in this group [Figure 3].

DISCUSSION

In this study, we evaluated the level of MMP-9 in the GCF before and after RCT in teeth with acute apical periodontitis, chronic apical periodontitis, and in healthy teeth. The results indicate that the local levels of MMP-9 increased in the two experimental groups with pulp and periapical inflammation and decreased following RCT.

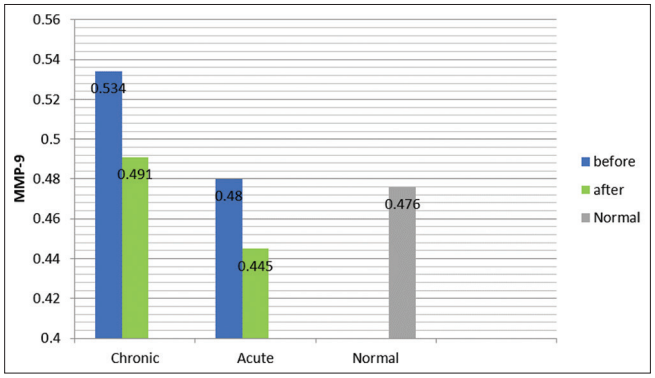

Figure 1: Matrix metalloproteinase-9 level comparison of the study groups.

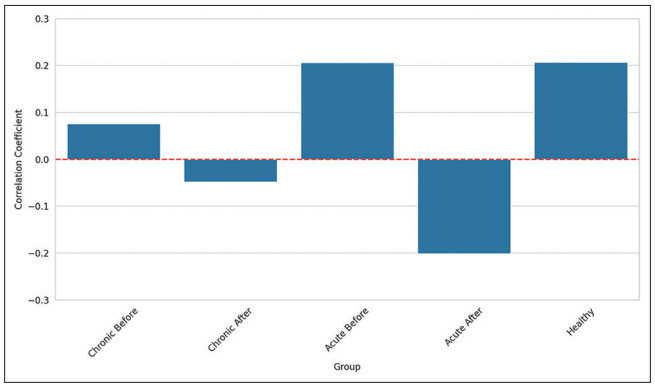

Figure 2: Correlation between gender and matrix metalloproteinase-9 levels.

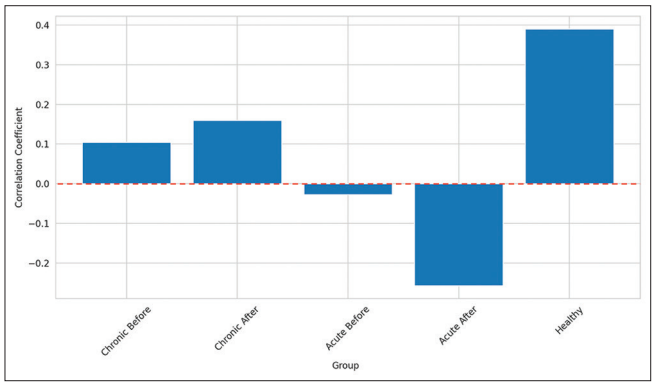

Figure 3: Correlation between age and matrix metalloproteinase-9 levels.

Various studies investigated the levels of MMP-9 to track the changes in pulp and periapical inflammation status.[11] For instance, Ballal *et al.* reported that the up-regulation of MMP9 in pulp wound has been known as a signature element for the gene expression in irreversible pulpitis.[12] Moreover, MMP-8 is suggested as one of the best diagnostic biomarkers for periodontal diseases and showed a high specificity and sensitivity on disease severity in a point-of-care mouthwash assessment.[13] Furthermore, Tsai *et al.* showed that the expression of MMP-9 mRNA in the pulp of teeth with inflammation increased compared to healthy teeth.[14]

Under normal conditions, the healthy periodontal ligament is protected from the proteolytic attack of MMPs by TIMPs. In chronic periodontitis, the level of TIMP is low and therefore, insufficient to inhibit MMPs. In this study, before the treatment, there was no significant difference between the MMP-9 level of the normal group and the acute periodontitis group. In contrast, there was a notable difference between the normal and chronic periodontitis groups. This can be due to the nature of the inflammatory response in acute versus chronic conditions. Acute apical periodontitis involves a rapid and transient inflammatory response, where MMP-9 levels may not have had sufficient time to rise significantly above those in healthy teeth.[15] In contrast, chronic apical periodontitis is characterized by prolonged and sustained inflammation, leading to consistently higher MMP-9 levels due to ongoing tissue remodeling and destruction.[16]

Regarding the changes in MMP-9 level after RCT, similar to our study, Shin *et al.* reported that the level of MMP-8 and substance P were measured in GCF before, during, and after RCT, and a decrease in the amount of inflammatory cytokines was observed after the treatment as a result of the reduction of inflammation.[17] Furthermore, Al-Abdulla *et al.* evaluated the serum level of MMP2, 2 years following nonsurgical root canal re-treatment and peri-apical surgery, and found that it can be a potential biomarker of inflammation indicating the successful treatment of chronic apical periodontitis.[18] In addition, Akbal Dincer *et al.* compared neurokinin A,

substance P, interleukin-8, and MMP-8 levels in healthy and symptomatic irreversible pulpitis, finding significant increases in these biomarkers in GCF of symptomatic teeth using ELISA technique, with decreases after pulp removal.[19]

In contrast to our study, Ahmed *et al.* reported that after the RCT procedure, the amount of MMP-9 in symptomatic periapical periodontitis lesion sections was considerably higher than in asymptomatic periodontitis; however, this amount did not differ notably in acute and chronic groups after the treatment in our study.[1] Moreover, Gusman *et al.* concluded that the level of MMP-1, MMP-2, and MMP-3 in acute pulpitis was higher compared to the healthy opposite tooth of the same person.[20] In addition, Victor *et al.* reported that the amount of MMP-9 in smokers with chronic periodontitis is higher than that of nonsmokers with chronic periodontitis.[5]

In contrast to our study, in the study of Zehnder *et al.*, it was shown that the amount of MMP-9 was detectable only in teeth with symptomatic pulpitis and could not be measured in healthy teeth.[6] This can be due to the different sampling methods and measuring the dentinal fluid instead of GCF. Different sources have been used for sampling local biomarkers such as apical lesions, periodontal ligament, gingival fluid, dentinal fluid, pulp tissue of extracted teeth, periapical exudate, and saliva.[1,5] Pattamapun *et al.* assessed MMP-2 levels in root-canal exudates during RCT and it notably decreased in teeth with necrosis after treatment.[21] Getting samples from root canal exudates can be highly influenced by instrumentation and chemical debridement that leads to the removal of residual microorganisms. Ahmed *et al.* figured out that, the possibility of measuring MMP in the dentinal fluid is low.[1] Also regarding getting samples from apical lesions, periapical surgery to remove periapical granuloma and tooth extraction to remove pulp tissue for this purpose is not ethically acceptable and may not be accepted by patients. Therefore, GCF was chosen as our study sample since it is a simple and noninvasive method and it is more frequently used in studies.

In our study, each canal was irrigated with 2 mL of 2.5% sodium hypochlorite (NaOCl, Cerkamed, Poland), and a final irrigation with 5 mL of distilled water (Farazdental, Iran) was performed. Higher concentrations of NaOCl (e.g., 5% and 5.25%) have been shown to further decrease MMP activity. Previous studies have demonstrated that while NaOCl and chlorhexidine reduce MMP expression in radicular dentin, EDTA can increase MMP levels.[22,23] Therefore, the use of 2.5% NaOCl aligns with these findings. By adhering to these standardized procedures, we aimed to minimize variability and focus on the specific outcomes related to the conditions under investigation.

To collect samples, different tools can be used such as PVDF membrane (polyvinyl fluoride filter membrane), medium size paper cone, and filter paper strip (standard filter paper tape).[2,24] The present study used a medium-sized paper cone due to its availability and easier use. Similar to Shin *et al.* study, a number of paper cones were placed in the tooth sulcus for 30 s and then placed in Tris HCL pH: 7.5 buffer solution before transferring to the laboratory.[17]

In order to analyze the MMP-9 of the samples, several laboratory analyses such as Zymography, Densitometry, ELISA, and Immuno Western Blot can be used.[10,25] In this study, due to the high accuracy of the ELISA, this test was used for laboratory tests.[26] Avellan *et al.* measured the molecular forms of MMP-8 in the GCF of stimulated and nonstimulated teeth by Western immunoblot, and MMP-8 levels by quantitative immunofluorometric assay and demonstrated elevations in mesenchymal type MMP-8 isoforms in stimulated teeth with pulpal pain.[24] In contrast with these findings, Kritikou *et al.* found no significant difference in the level of MMP9 in healthy and symptomatic irreversible pulpitis using the ELISA technique.[27]

Future research should incorporate different groups using various irrigants, concentrations, obturation techniques, and sealer types to compare their effects on MMP-9 levels. Examining these variables separately could provide deeper insights into how specific clinical procedures influence outcomes. Furthermore, a larger sample size is needed to provide a more precise correlation between the study variables.

CONCLUSION

Our results indicate that MMP-9 levels in GCF rise during periapical inflammation but decline following endodontic treatment. MMP-9 is a potential diagnostic biomarker for both pulp and periapical inflammation, offering insights into these clinical conditions and guiding future treatment approaches.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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