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Matrix Metalloproteinase 9 Level Changes in the Gingival Crevicular Fluid Samples of Teeth with Symptomatic and Asymptomatic Irreversible Pulpitis: A Prospective Cohort Study

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Abstract

Background and objectives: This article delve into the valuable insights provided by molecular markers, particularly Matrix Metalloproteinase 9 (MMP-9), in the context of pulpal diagnosis. It emphasizes the challenges associated with current diagnostic tools and advocates for enhanced methods to ensure a reliable diagnosis. Elevated MMP-9 levels emerge as potential biomarkers not only for pulpal diseases but also for periodontal and cardiovascular conditions. The exploration extends to molecular alterations and diagnostic biomarkers in gingival crevicular fluid, shedding light on promising avenues for dental diagnosis and customized treatment approaches.

Materials and methods: Gingival crevicular fluid (GCF) samples were collected from 56 eligible patients using medium-sized paper points. A second set of GCF samples was collected two weeks post- root canal treatment. Normal samples were obtained from patients referred for routine dental check-ups. GCF samples were transferred to sampling tubes with Tris HCL pH = 7.5 buffer. MMP-9 levels were measured using ELISA.

Results: Statistical analysis revealed a significant decrease in MMP-9 levels in GCF after root canal treatment for both symptomatic and asymptomatic irreversible pulpitis (P-value < 0.05). The study identifies an increase in MMP-9 during pulp inflammation. Subsequently, MMP-9 levels decrease in the gingival crevicular fluid after root canal treatment, indicating the resolution of inflammation.

Conclusion: Precision in evaluating and measuring enzymes associated with pulp tissue destruction in the local gingival crevicular fluid is achievable through a precise method. The examination of MMP-9 in gingival crevicular fluid can serve as a valuable tool for monitoring inflammatory activity and assessing the success of treating teeth with symptomatic and asymptomatic irreversible pulpitis.

Keywords: Gingival crevicular fluid; Matrix metalloproteinase-9; Pulpitis; Root canal therapy.

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1. Introduction

Irreversible pulpitis is a clinical determination based on subjective and objective findings, denotes an inflamed pulp incapable of self-healing, necessitating root canal

treatment (1). According to the American Association of Endodontists (AAE) Consensus Conference Recommended Diagnostic Terminology, symptomatic irreversible pulpitis is characterized by acute pain upon exposure to thermal stimulus, persistent pain after stimulus removal, spontaneous pain and referred pain. Over-the-counter analgesics often do not provide relief. Diagnosing symptomatic irreversible pulpitis can be challenging, especially when inflammation hasn't extended to periapical tissues, resulting in a lack of pain or discomfort during percussion. In such cases, assessing dental pulp condition heavily relies on dental history and thermal testing. Asymptomatic irreversible pulpitis, despite lacking clinical symptoms, typically demonstrates normal responses to thermal testing (2).

Accurate diagnosis of pulp and periapical diseases is pivotal for informed treatment decisions. The primary diagnostic tools for pulpal disease include clinical findings, examinations, pulp sensitivity tests and X-rays. However, these methods lack complete reliability. Clinical diagnosis offers a provisional judgment with potential errors due to incomplete correlation with histological conditions. While histological examination provides a definitive diagnosis, it's impractical before treatment (3). The text underscores the need for enhanced methods to ensure a dependable diagnosis.

Matrix metalloproteinases (MMPs) are vital zinc-dependent endopeptidases that exhibit significant importance (4). The enzymes fulfill discrete roles in biological processes, encompassing modifications to the extracellular matrix (ECM), stimulation of angiogenesis, facilitation of cellular migration across blood vessels and assistance in the development of immune cells. The dysregulated control and heightened expression of specific members within the MMP family can have an impact on these mechanisms, thereby contributing to a wide array of pathological conditions, including but not limited to neurodegeneration, arthritis, cardiovascular disorders, fibrosis and cancer (5).

Matrix Metalloproteinase 9 (MMP-9), or gelatinase B, is a proteolytic enzyme secreted by various cell types such as neutrophils, fibroblasts and macrophages. The active form of MMP-9 plays a crucial role in degrading the extracellular matrix, maintaining proper balance, contributing to the breakdown of the pulpal tissue (6). MMP-9 also produces bioactive proteins, including growth factors, chemokines and cytokines. Degrading type IV collagen, MMP-9 interferes with basement membranes connected to tumor infiltration and metastasis (7). While MMP-9 has been identified in symptomatic pulpitis, it is absent in the healthy dental pulp (8).

Individuals diagnosed with periodontitis demonstrate notably increased levels of MMP-9 in their serum (9). One valid biomarker for the diagnosis of patients with periodontal diseases is elevated MMP-9 levels in gingival crevicular fluid and the periodontal ligament. Perhaps, these levels decline with efficient periodontal therapy. Given this information, one could hypothesize that MMP-9 acts as a regulator of periodontal lesions (4). Elevated circulating levels of MMP-9 in patients with periodontal disease may function as an early marker for general inflammation (10). Measuring MMP-9 levels in GCF allows researchers to assess inflammatory activity and monitor the success of treating teeth with symptomatic and asymptomatic irreversible pulpitis. These findings hold promise in diagnosing pulp inflammation severity, and facilitating customized treatment approaches.

2. Materials and methods

2.1. Study Population

In this study, 57 molars were selected from 57 patients referred to the Islamic Azad University of Medical Sciences, School of Dentistry, Endodontics Department, and the Oral Medicine Clinic during the academic year 2022-2023. The sample size was determined using the Bonferroni formula as follows, and based on the information obtained from a study (11) on MMP levels in the gingival crevicular fluid in the target groups, with $n1 = n2 = n3 = 15$. The sample included 38 affected teeth and 19 healthy control teeth. Of the affected molars, 19 were diagnosed with symptomatic irreversible pulpitis, and 19 with asymptomatic irreversible pulpitis. All cases exhibited a positive response to the pulp vitality test. Patients with symptomatic cases reported a history of pain, while those with asymptomatic cases did not report pain symptoms. The control teeth were healthy, unrestored and free from caries.

None of the patients had general diseases that might affect healing, and they had not taken antibiotics or pain medicine including NSAIDs within 1 week before or during the therapeutic duration. Subjects experiencing non- dental inflammatory diseases, ulcers, systemic diseases, long-term use of anti-inflammatory drugs and smoking were excluded.

2.2. Patients' subjective symptoms and clinical examination

Patients with apical acute periodontitis primarily reported pain when the affected tooth was percussed. They also experienced discomfort while chewing, biting, and occasi-

-nally had spontaneous pain. Their response to pulp viability tests varied, and radiographic images typically revealed slight widening of the periodontal ligament. In contrast, teeth with chronic apical periodontitis showed no clinical symptoms and were unresponsive to pulp vitality tests. Radiographs of these teeth often displayed apical radiolucency. While these teeth were generally not sensitive to chewing pressure, patients did perceive a distinct sensation during percussion testing.

2.3. GCF Sampling

The target area was meticulously isolated and dried using gentle airflow. Sampling involved using four #30 paper cones inserted into the gingival sulcus on buccal, lingual and interproximal surfaces. Clinical procedures were carried out under local anesthesia and rubber dam isolation to ensure aseptic conditions, all performed by a single operator. Two weeks post- root canal treatment for patients with symptomatic and asymptomatic irreversible pulpitis, the procedure was repeated, and any contaminated samples were discarded. Control teeth underwent the same actions. The collected samples were stored in a sample tube containing Tris- HCl buffer at pH 7.5. They were then placed on a rotator for 3 hours, and stored at -20 degrees Celsius, and then transferred to the laboratory. MMP-9 levels were measured in µg/µL using an Enzyme-Linked Immunosorbent Assay (ELISA) with a Human MMP-9 Elisa Kit from Sigma Aldrich Inc., based in Missouri, USA. This process was repeated two weeks post-root canal treatment, excluding contaminated samples and control teeth underwent similar procedures.

2.4. Statistical Analysis

One- Way ANOVA was employed for the statistical assessment using IBM SPSS statics for Windows (Version 22.0. Armonk, NY: IBM Corp)

3. Results

After obtaining all the samples, the levels of MMP-9 were assessed using the ELISA technique, and the obtained data underwent statistical scrutiny (Table 1). According to the statistical analysis, the data followed a normal distribution. One- Way ANOVA was employed for the statistical assessment. The findings indicated no notable variance in MMP-9 values before root treatment among the three groups (P-value = 0.325. Nevertheless, a noteworthy distinction was observed in MMP-9 levels after treatment between the investigated groups (P-value = 0.011). Subsequent pairwise comparisons post-treatment unveiled significant distinctions solely between the normal and symptomatic groups (P-value = 0.012).

The outcomes affirmed a substantial contrast in MMP-9 values pre- and post- treatment for both symptomatic and asymptomatic groups individually (P-value = 0.0001). In the Asymptomatic cohort, the average MMP-9 diminished by 0.06 subsequent to the intervention, whereas in the Symptomatic cohort, this reduction amounted to 0.07.

4. Discussion

The existing criteria utilized to evaluate pulp condition rely on clinical and radiographic alterations. Given the limitations of traditional diagnostic approaches, there is a clear necessity for modern methods to identify changes. While histology is the gold standard for determining the inflammatory state of pulp tissue, it is impractical before root canal treatment. The current investigation proposes that MMP-9 functions as a valuable indicator for detecting inflammatory conditions in dental pulp.

Analyzing MMP-9 GCF across different pulp and periapical situations, encompassing symptomatic and asymptomatic irreversible pulpitis, as well as healthy teeth pre and post root canal treatment, highlights its potential diagnostic importance. Our findings indicate that MMP-9 levels exhibit a local increase in the gingival crevicular fluid of a tooth with pulp inflammation, subsequently decreasing after root canal treatment.

ELISA stands as a highly sensitive and widely employed

Table 1. Statistical characteristics of MMP-9 before and after root canal treatment in the three studied groups

MMP-9 level / Group	Mean ± SD		P-Value
	Before treatment	After treatment	
Asymptomatic irreversible pulpitis	0.496 ± 0.042	0.436 ± 0.039	0.0001
Symptomatic irreversible pulpitis	0.494 ± 0.047	0.424 ± 0.066	0.0001
Healthy	0.476 ± 0.046	0.476 ± 0.046	
P-Value	0.325	0.011	

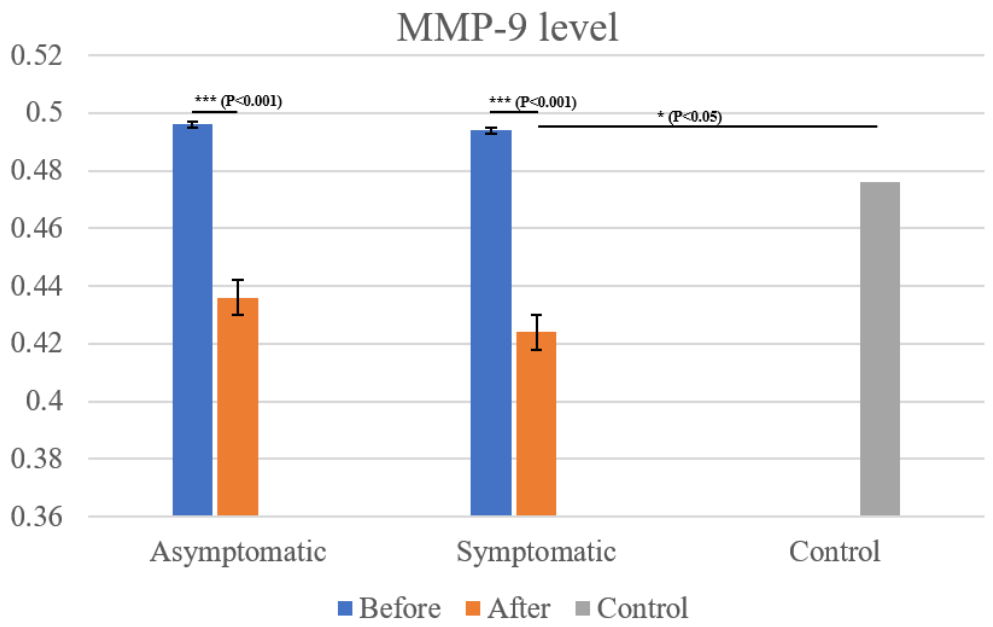

Figure 1. MMP-9 level comparison between the study groups.

biochemical diagnostic method for analyzing fluid-based biomarkers in periodontal research. Its advantages encompass high throughput and reproducibility even with minute samples. However, it is essential to avoid asserting that ELISA's superiority over alternative techniques such as radioimmunoassay, Immunohistological staining, western blotting, Zymography and Densitometric which are also utilized for characterizing GCF content. Commercial ELISA kits, nonetheless, offer meticulous protocol optimization, validation characteristic determination and the acquisition of an appropriate standard (12-14). In this study, GCF biomarker levels were assessed using commercial ELISA kits, adhering to the manufacturer's guidelines according to a study by Gur *et al.* (15-17).

Various MMP sampling sources have been utilized in different studies, including blood from exposed dental pulp (18), periapical lesions (19), whole pulp tissue (12, 20) and saliva samples for genomic DNA (11) Dentinal Fluid (8). GCF was selected as the study sample due to its frequent use in research, easier sampling, and less invasiveness, similar to the approaches by Heidari *et al.* (21).

Different devices are available for sample collection, such as Polyvinylidene Fluoride filter membrane (PVDF) membrane (8) and standard filter paper strips (21-23). We opted for sterilized paper points, following Shine et al. study, who used paper points to measure MMP-8 levels in GCF (17). The consistent longitudinal data from control sites support the appropriate use of paper points as GCF collection devices. Similar to our study, Avellan *et al.* observed that stimulating pulp tissue and inducing local inflammation in the tooth increases MMP levels in gingival crevicular fluid (22).

Dincer and colleagues utilized teeth on the opposite side of the mouth belonging to the same participants, whereas our investigation employed a separate control group consisting of individuals who were seeking regular dental examinations (24).

Shin *et al.* discerned a decline in the levels of MMP8 and Substance P after the administration of root canal treatment, which was causally connected to the abatement of inflammation within the dental structure following the therapeutic intervention. In our study, we observed a minimal variance in MMP-9 levels after treating teeth with symptomatic and asymptomatic irreversible pulpitis compared to the normal group (17).

In Zehnder *et al.* study, MMP-9 levels were observed exclusively in teeth exhibiting symptomatic pulpitis and not in healthy teeth. In contrast, our study detected MMP-9 in all groups, albeit at different levels. Notably, in over half of the symptomatic teeth in their study, MMP-9 levels were below the detection limit. The variation in results might be attributed to differences in sample collection methods between the studies (8).

The necessity for novel diagnostic tools for pulp is becoming increasingly apparent. Approaches grounded in molecular principles hold significant potential in this context. By offering more accurate diagnoses, these molecular-based approaches will have a transformative

effect on the decision- making process of dental practitioners and will improve patient evaluation and treatment by moving toward personalized therapies. In a comprehensive review by Zanini *et al.*, they concluded that clinical irreversible pulpitis is associated with specific levels of expression of inflammatory mediators. Moreover, they noted an elevation in the MMP-9 level in response to inflammation (25). Their findings regarding the elevation of mediator levels in irreversible pulpitis align with our own.

Zhou *et al.* observed increased MMP-9 levels in the serum, pulp tissues, and saliva of pediatric patients experiencing pulpitis and Helicobacter pylori (Hp) infection in the stomach. This suggests that HP infection might exacerbate pulpitis by increasing MMP-9 levels in saliva, pulp tissues, and blood. MMP-9 emerges as a potential marker for detecting HP infection in children with pulpitis. This aligns with our findings of elevated MMP-9 levels linked to pulpitis (26).

Chen *et al.* studied combined datasets of pulpitis tissue, seeking to understand methods for diagnosing or treating varying levels of pulpitis inflammation. They identified that MMP9 levels in the blood samples of irreversible pulpitis significantly raised compared to those in blood samples from asymptomatic or reversible pulpitis teeth, identifying it as a potential biomarker candidate for diagnosing pulpitis. While their results aligned with ours, the methodologies employed differed (27).

It is suggested to investigate the level of MMP-9 in other dental diseases and cases of dental trauma in future investigations. Also, future studies should explore the impact of different irrigants, concentrations, obturation techniques, and sealer types on MMP-9 levels by comparing multiple groups. Investigating these factors individually could offer a more detailed understanding of how specific clinical practices affect outcomes. Additionally, increasing the sample size would enhance the accuracy of the correlations between the variables studied.

5. Limitations

It is suggested to investigate the level of MMP-9 in other dental diseases and cases of dental trauma in future investigations. Additionally, increasing the sample size would enhance the accuracy of the correlations between the variables studied.

5. Conclusion

The objective of this study was to compare MMP-9 levels before and after root canal therapy in symptomatic and asymptomatic irreversible pulpitis, aiming to enhance comprehension of inflammatory processes. The findings suggest that MMP-9 levels increase in the gingival crevicular fluid of a tooth with pulp inflammation and subsequently decrease after root canal treatment.

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Ethics

Both the control and patient groups received comprehensive information regarding the research protocol. This paper was extracted from the thesis approved by the Research Ethics Committee of Tehran Islamic Azad University of Medical Sciences (Thesis code: 25157), it also followed the guidelines of the Helsinki Declaration.

Author contributions

Amirabbas Moshari, Mehdi Vatanpour: Supervision, Validation, Conceptualization.

Sarvin Mesbahian, Mohammad Mahdi Khanmohammadi, Nozhan Azimi: Writing- Review & Editing, Writing Original Draft, Formal analysis, Software, Methodology.

Shiva Shirvani Samani: Investigation, Writing- Original Draft.

Conflict of interest

The authors declare no conflict of interest.

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