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Original Article

Matrix metalloproteinase 8 production of cariously-exposed irreversible pulpitis

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KEYWORDS
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Abstract *Background/purpose:* Matrix metalloproteinase 8 (MMP-8) is a major enzyme released by neutrophils and may serve as a potential biomarker for pulpal inflammation. This study aimed to compare MMP-8 levels in normal pulp and cariously-exposed pulp diagnosed with irreversible pulpitis, including both asymptomatic irreversible pulpitis (AIP) and symptomatic irreversible pulpitis (SIP) and to assess the association between clinical factors and MMP-8 concentrations in the pulpitis group.
Materials and methods: Pulpal blood samples were collected following caries removal in pulpitis cases or intentional pulp exposure in the normal pulp group. Both active and total forms of MMP-8 were measured. Clinical factors that might affect the concentrations of MMP-8 in the pulpitis group (AIP and SIP), were included to determine theircorrelations.
Results: Fifteen teeth diagnosed with irreversible pulpitis (AIP and SIP) and five sound premolars (normal pulp) were included. Higher concentrations of MMP-8 were observed in both AIP and SIP groups (AIP: active $=$ 18.40 $\pm$ 3.04 ng/mL, total $=$ 36.79 $\pm$ 7.88 ng/mL; SIP: active $=$ 16.06 $\pm$ 3.10 ng/mL, total $=$ 32.02 $\pm$ 6.12 ng/mL) compared to normal pulps (active $=$ 4.63 $\pm$ 0.96 ng/mL, total $=$ 7.17 $\pm$ 1.41 ng/mL). No significant difference was found between AIP and SIP. The presence of radiographic periapical pathology was significantly associated with increased MMP-8 concentrations in the pulpitis group.
Conclusion: Irreversible pulpitis exhibited elevated MMP-8 levels regardless of pulpal pain symptoms. MMP-8 concentrations were significantly higher in cases with periapical pathology, supporting its potential role as a bioactive marker for pulpal inflammation.

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Introduction

According to the American association of endodontists (AAE) diagnostic terminology, a deep caries lesion with anticipated pulp exposure following caries removal, or a carious exposed pulp tooth is diagnosed to irreversible pulpitis regardless of clinical symptoms.[1,2] This inflamed pulp was not believed to be treated with vital pulp therapy (VPT). Thus, pulpectomy is recommended.[3] However, current clinical studies have demonstrated that irreversible pulpitis could be managed with VPT.[4–8] This suggests that current pulpitis terminologies may not reliably reflect the true extent and severity of pulpal inflammation. In cases of mild to moderate pulpitis, such as those presenting with prolonged responses to thermal stimuli lasting less than 20 s, pain that can be relieved by analgesics, treatment options may include pulp capping, partial pulpotomy, or full pulpotomy.[9] However, in cases of severe pulpitis where pain is unrelieved by analgesics and interferes with daily function, full pulpotomy or pulpectomy should be considered.[9]

The assessment of whether a surface pulpal wound or an excisional pulpal wound exhibits inflammation that can be reversible or requires further tissue removal to the reversible area remains ambiguous. Although bleeding time evaluation has long been recommended as a classic clinical assessment tool, spontaneous haemostasis can be achieved within 5–10 min by sodium hypochlorite (NaOCl) solution.[10] This method is inherently subjective and may not accurately reflect the true extent of pulpal inflammation.[11] Various candidate biomarkers represent a promising alternative for assessing inflamed pulp wounds, including cytokines, neuropeptides, and enzymes such as interleukin (IL)-1β,[12,13] IL-8,[12,14] TNF-α,[12,14] substance P,[12,15,16] calcitonin gene-related peptide (CGRP)[12,15] and MMP-9 [17] have been showed to be elevated in teeth with pulpitis from dental caries, in both asymptomatic irreversible pulpitis (AIP) and symptomatic irreversible pulpitis (SIP).

Histological studies have shown that cariously-exposed pulp teeth demonstrated significant accumulation of neutrophils under pulpal wound area. Over time, the accumulation of neutrophils leads to the release of various enzymes, resulting in nonspecific tissue destruction.[18] Among these enzymes, MMP-8, also known as neutrophil collagenase, is of particular interest. MMP-8 primarily targets collagen, a major component of the extracellular matrix of dental pulp tissue.[19] Thus, MMP-8 should be considered a biomarker candidate for assessing the inflammation stage of the surface or surgical pulpal wound. Dentin debris from teeth diagnosed with pulpitis contained higher levels of MMP-8 compared to those with normal pulp.[20] However, the study of MMP-8 levels from direct exposed pulp site from caries is limited. Therefore, the objective of this study was to determine amount of MMP-8 at the surface pulpal wound from both AIP and SIP from caries.

Materials and methods

This study was approved by the human research committee of Thammasat University. The certification number was COA no. 109/2567. Patients were asked to sign informed consent before participating.

Patient selection and clinical pulp diagnosis

According to the previous study,[20] G*power program was used. The effect size was 0.61. The alpha error and power were 0.05 and 0.95, respectively. The number of samples in each group was calculated at 15. The pilot study revealed that MMP-8 levels were low and relatively stable in teeth with normal pulp (data not shown). Therefore, the sample size for this group was reduced to five, which was still sufficient for statistical analysis.

Healthy patients were selected from both sex at aged 18–65 years old. No nonsteroidal anti-inflammatory or narcotic drugs used for 24 h prior blood collection. The normal pulp group were sounded premolar teeth that planned to extract for orthodontic treatment. The pulpitis groups were restorable deep carious teeth. Patients were comprehensive history taking of pain history. Sensitivity test such as thermal and electrical test were used to reproduce the patients's symptoms and confirm the pulpal vitality. Percussion and palpation tests were used for periapical tissue inflammation examinations. The periapical radiograph was also taken for periapical tissue pathology examination. The pulpal diagnosis followed the AAE diagnostic terminology.[1,2] SIP was characterized by vital inflamed pulp presenting with signs and symptoms, including lingering thermal pain or spontaneous pain. AIP was characterized by a vital pulp vital without clinical symptoms, but exposed pulp was expected by deep caries and/or caries excavation. The exclusion criteria included teeth that blood could not be collected at pulpal exposure site, teeth with necrotic pulp were identified upon access opening, teeth that could not be isolated with a rubber dam. A total of 30 teeth met the criteria (Fig. 1). The demographic characteristics of the 30 included teeth are presented in Table 1.

Clinical procedures and blood sample collection

Blood collection

Teeth were anesthetized with 2 % lidocaine solution with 1:100,000 epinephrine (Septodont, Saint-Maur-des-Fosses,

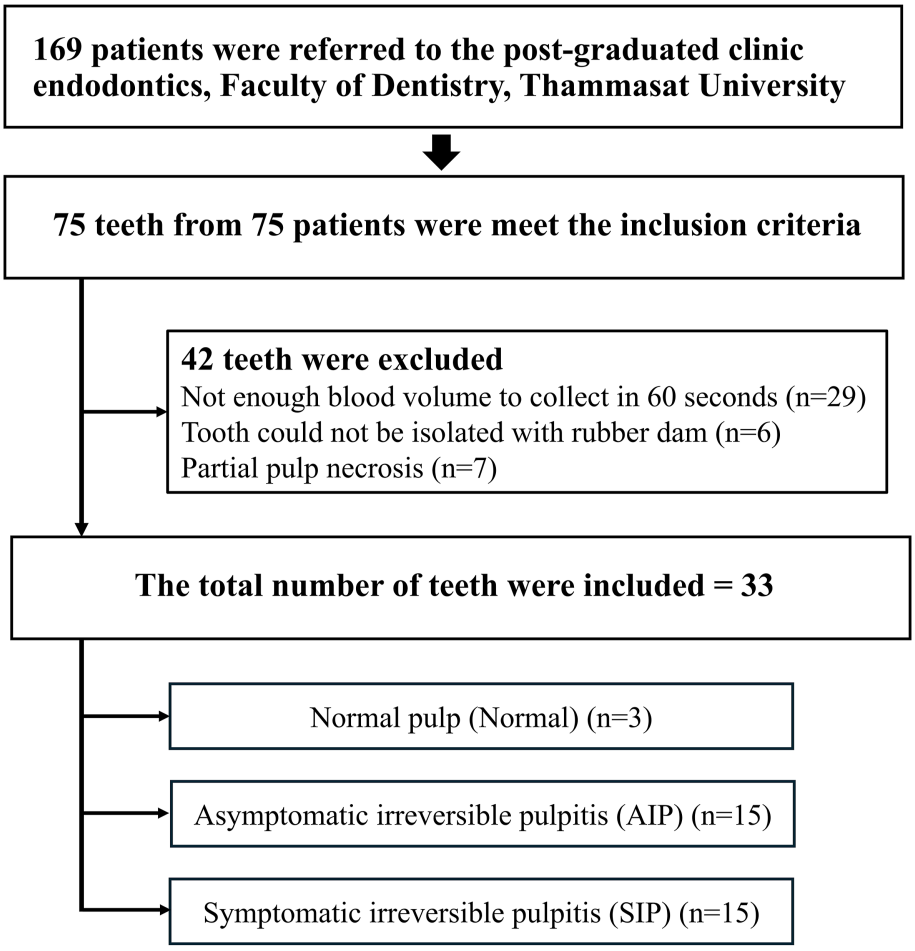

Figure 1 Diagram of the screening and selection process of included teeth.

France). Teeth were isolated by a rubber dam. Surrounded carious lesions were removed with high speed round surgical burs. Both hard and soft carious lesions near the pulp exposure site were carefully removed by round low speed carbide surgical burs until exposed pulp tissue was observed. The cotton pellets were placed at the pulpal exposure site for 60 s. The obtained blood was transferred into 1 mL of normal saline solution with blood collection heparin-coated tube (Thermo scientific, Waltham, MA, USA) (Fig. 2).

Management of dental pulp following blood collection

All teeth in the pulpitis group could not be restored with simple filling restorations due to extensive loss of tooth structure. Pulpectomy was performed in cases where the root canal anatomy was not complex and sufficient time was available. The working length was determined using an apex locator (Root ZX, J Morita Corp, Osaka, Japan) and was confirmed by periapical radiographs. Reciproc Blue® files (VDW, Munich, Germany) were used up to size R25 or R40 in larger canals. Throughout the procedure, 2.5 % Sodium hypochlorite (NaOCl) solution was used for root canal irrigant. Calcium hydroxide paste (Ultracal™ XS, South Jordan, UT, USA) was applied as an intracanal medicament. The tooth was subsequently restored with a semi-permanent composite resin (Filtek™ Z250, 3M ESPE, Seefeld, Germany) and scheduled for definitive root canal treatment later. For teeth with complex anatomy and/or insufficient time, full coronal pulpotomy was performed instead. These cases were also restored with composite resin and scheduled for definitive root canal treatment.

Serum preparation and enzyme-linked immunosorbent assay

Tubes with blood sample were centrifuged at 2000 g at 4 °C for 10 min. The supernatant was carefully collected and designed as a serum. The serum was immediately transferred into a clean polypropylene tube for 0.5 mL. Active form of MMP-8 (ActiveMMP-8) concentration was analyzed using human MMP-8 activity assay (QuickZyme Biosciences, Leiden, Netherland) according to the manufacturer's instructions (Fig. 2). Briefly, goat's F(ab)'2 anti-MMP-8 was used for pre-coated 96-well assay plate and incubated at 37 °C for 2 h. After washing with wash buffer, 100 μL of samples were added to the plate and covered with a lid, then, incubated at 4 °C overnight. After washing, 50 μL of assay buffer was added and the plate was incubated at 37 °C for 1

Table 1 Demographic data of included teeth.

Attribute	n	%
Categories		
Age		
18–30	16	53.34
31–65	14	46.66
Total	30	100.00
Sex		
Male	14	46.67
Female	16	53.33
Total	30.	100.00
Tooth type		
Premolar	1	3.33
Molar	29	96.67
Total	30	100.00
Location		
Maxillary	15	50.00
Mandibular	15	50.00
Total	30	100.00
Pulpal diagnosis		
Asymptomatic irreversible pulpitis	15	50.00
Symptomatic irreversible pulpitis	15	50.00
Total	30	100.00
Normal pulp	5[a]	
Apical lesion size		
Normal periapical tissue	8	26.67
Widened periodontal ligament	12	40.00
Periapical lesion	10	33.33
Total	30	100.00
Apical pain		
None	16	53.30
Pain	14	46.70
Total	30	100.00

[a] For control group or base line to compare matrix metalloproteinase 8 (MMP-8) concentration to irreversible pulpitis (asymptomatic irreversible pulpitis and symptomatic irreversible pulpitis).

h before adding 50 μL of detection reagent and shaken for 20 s, then incubated for an additional 6 h. The plate was read at wavelength of 405 nm using a microplate reader (Multiskan SkyHigh, Thermo scientific). To measure the total MMP-8 production (TotalMMP-8), 50 μL of p-aminophenyl mercuric acetate (APMA), a pro-MMP8 activator, was added to serum sample following overnight incubation. Then, the measurements were conducted with same methods of ActiveMMP-8. The optical density (OD) was adjusted by subtracting the blank value. The OD values were then fitted to a standard curve to determine the amount of MMP-8, which was subsequently converted into Active and TotalMMP-8 concentration (ng/mL).

Statistical analysis

Kruskal–Wallis test was applied to compare Active and TotalMMP-8 concentration among the normal pulp, AIP and SIP groups. Post-Hoc analysis was performed using the Dunn–Bonferroni test. Clinical parameters were evaluated for correlation with both Active and TotalMMP-8 concentration using Spearman's correlation coefficient within the only irreversible pulpitis (pooled data of AIP and SIP) group. Data analysis was conducted using SPSS version 22 (IBM, Armonk, NY, USA), with the significance level set at $P < 0.05$.

Results

Both ActiveMMP-8 and TotalMMP-8 concentration were higher in the AIP (ActiveMMP-8: Median = 12.32 ng/mL, Mean ± SD = 18.40 ± 3.04 ng/mL; TotalMMP-8; Median = 24.16 ng/mL, Mean ± SD = 36.79 ± 7.88 ng/mL) and the SIP group (ActiveMMP-8: Median = 13.05 ng/mL, Mean ± SD = 16.06 ± 3.10 ng/mL; TotalMMP-8; Median = 33.71 ng/mL, Mean ± SD = 32.02 ± 6.12 ng/mL) compared to the normal pulp group (ActiveMMP-8: Median = 3.17 ng/mL, Mean ± SD = 4.63 ± 0.96 ng/mL; TotalMMP-8; Median = 5.78 ng/mL, Mean ± SD = 7.17 ± 1.41 ng/mL). However, there was no statistically significant difference in MMP-8 levels between the AIP and SIP (Fig. 3A and B).

Clinical variables—including age, sex, tooth type, pulpal diagnosis, presence of periapical pain, and tooth location—showed no correlation both ActiveMMP-8 and TotalMMP-8 concentrations in the pulpitis group. Radiographic findings, categorized as normal periapical tissue (Normal), widened periodontal ligament (WP), and periapical lesion (PA), demonstrated a significant correlation with TotalMMP-8 levels (correlation coefficient = 0.37, $P = 0.02$). However, no significant correlation was observed between ActiveMMP-8 and radiographic pathology (correlation coefficient = 0.15, $P = 0.21$) (Fig. 4). To further investigate this association, the Kruskal–Wallis test was employed. ActiveMMP-8 concentrations did not significantly differ among the groups: normal periapical tissue (Median = 7.20 ng/mL, Mean ± SD = 11.21 ± 2.57 ng/mL), WP (Median = 12.62 ng/mL, Mean ± SD = 16.82 ± 3.69 ng/mL), and PA (Median = 12.81 ng/mL, Mean ± SD = 19.24 ± 4.05 ng/mL) (Fig. 3C). In contrast, teeth with WP (Median = 33.71 ng/mL, Mean ± SD = 34.22 ± 6.55 ng/mL) and PA (Median = 38.66 ng/mL, Mean ± SD = 46.43 ± 10.61 ng/mL) exhibited significantly higher in TotalMMP-8 concentrations compared to normal radiographic findings (Median = 10.2 ng/mL, Mean ± SD = 14.84 ± 4.07 ng/mL) (Fig. 3D).

Discussion

This study supported the hypothesis that MMP-8 serves as a potential biomarker of pulpal inflammation in cariously-exposed pulp teeth. MMP-8 raised significantly higher concentrations than in normal pulp. Significantly elevated concentrations of MMP-8 were observed in inflamed pulps compared to normal pulps. However, no significant difference in either ActiveMMP-8 and TotalMMP-8 levels was observed between AIP and SIP, suggesting that the presence or absence of pain may not be influenced by MMP-8 production.[16] These findings imply that MMP-8 primarily reflects the extracellular matrix degradation rather than nociceptive signaling. A significant association was

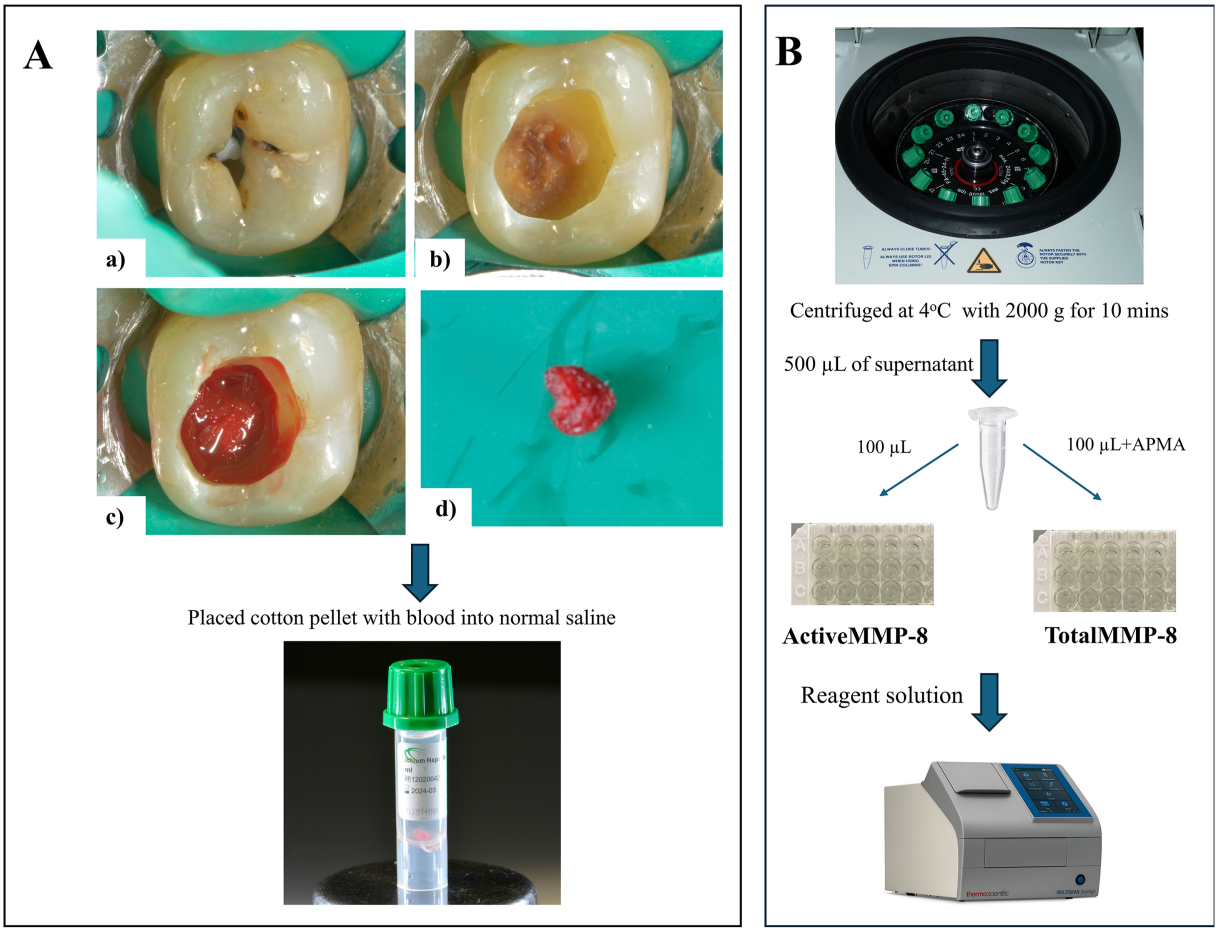

Figure 2 Sample collection, preparation and matrix metalloproteinase 8 (MMP-8) measurement. (A) Blood collection from the exposed pulpal wound surface: (a) before caries removal; (b) after removal of surrounding hard and soft caries; (c—d) bleeding from the pulp following caries removal and blood collection by using cotton pellet; (e) transfer sample into a collection tube. (B) Preparation of serum samples: for both active MMP-8 (ActiveMMP-8) and total MMP-8 (TotalMMP-8) assays, 100 µL of each sample was used; p-aminophenyl mercuric acetate (APMA) was added only for TotalMMP-8 measurement.

observed between MMP-8 levels and radiographic evidence of periapical pathology. This finding raises an intriguing possibility that MMP-8 may serve as a marker for the severity of periapical tissue destruction, not only within inflamed dental tissues affected by caries but also in cases with periapical involvement.[18] Given the complexity of pulp pathology, the integration of objective biomarkers into diagnostic procedures may enhance both the accuracy of diagnosis and the appropriateness of treatment decisions. Early research efforts have focused on distinguishing irreversible pulpitis from healthy pulp tissue, as well as differentiating SIP from AIP cases. These studies have employed various sampling methods, including whole pulp tissue,[16,21] dentin powder surrounding the exposed pulp,[20] saliva,[22] and crevicular fluid.[16,23] Notable biomarkers such as TNFα, IL-6, IL-8, CGRP, substance P and MMP-9 [13-15,17,24] have been identified as potential indicators of pulpal inflammation. However, the complexity of the relationship between biomarkers and clinical symptoms extends beyond simply associating specific markers with particular pulp diagnoses. The use of biomarkers to assess pulpal inflammation requires that the selected biomarkers facilitate clinical decision-making—specifically, whether to preserve the pulp or proceed with pulpectomy—when considered alongside clinical signs and symptoms. Furthermore, in cases where pulp preservation is indicated, biomarkers should help determine the optimal depth of pulp tissue removal necessary to promote pulp healing and effectively alleviate pain. It is noteworthy that this topic has been investigated in clinical studies, highlighting the potential application of biomarkers in conjunction with clinical symptoms to assess the true inflammatory status.

A previous clinical study reported that cases with successful full pulpotomy cases had a median MMP-9 concentration of 132.3 ng/mL, while failed cases requiring pulpectomy had significantly higher levels (512.4 ng/mL), reinforce the potential of biomarker-based diagnosis.[25] MMP-8 is another promising biomarker for assessing pulpal inflammation alongside MMP-9, as both enzymes are predominantly secreted by neutrophils during inflammatory responses.[26] Previous study has quantified MMP-8 levels in irreversible pulpitis, revealing significantly higher concentrations compared to normal pulp and reversible pulpitis.[20] Although this study measured MMP-8 in dentin powder

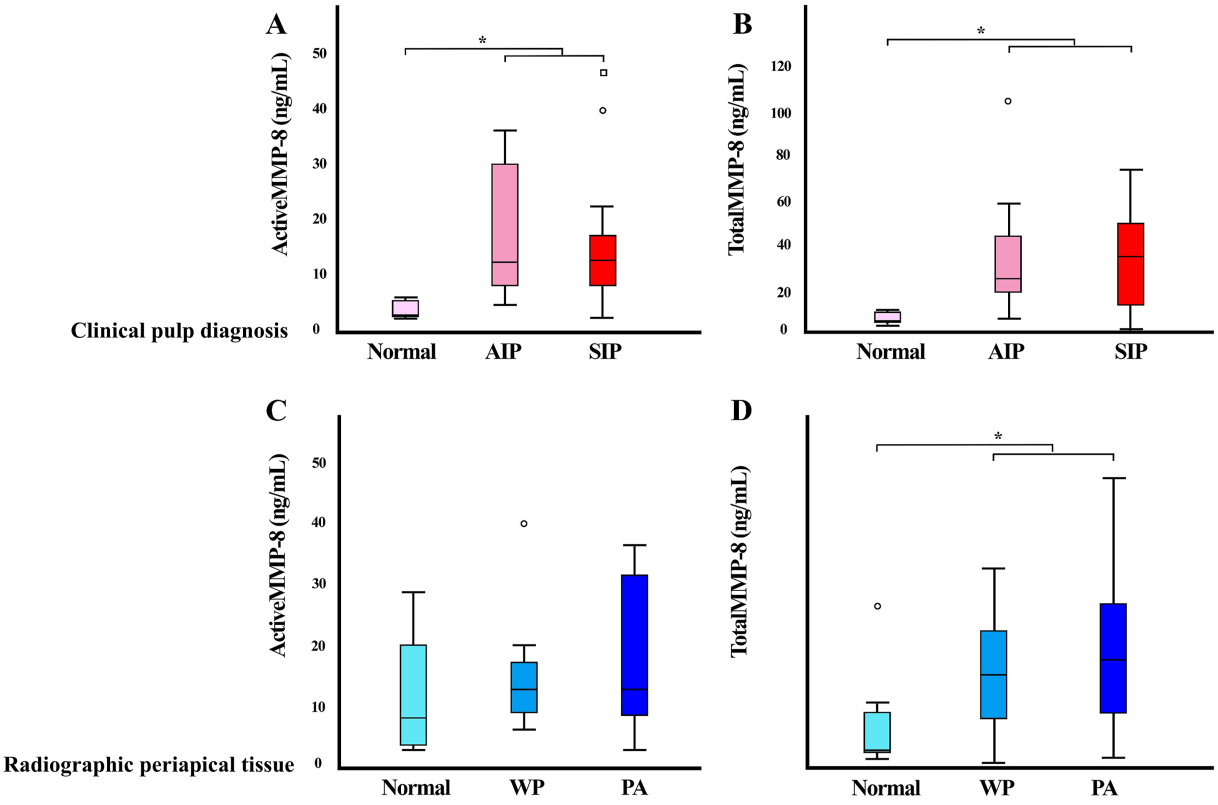

Figure 3 Concentrations of matrix metalloproteinase 8 (MMP-8). (A) Concentrations of active form of MMP-8 (ActiveMMP-8) according to pulpal diagnosis; normal pulp (Normal), asymptomatic irreversible pulpitis (AIP), symptomatic irreversible pulpitis (SIP). (B) Concentrations of total MMP-8 production (TotalMMP-8) according to pulpal diagnosis. C) Concentrations of ActiveMMP-8 from irreversible pulpitis with different radiographic periapical tissue findings; normal periapical tissue (Normal), widened periodontal ligament (WP), periapical lesion (PA). D) Concentrations of TotalMMP-8 from irreversible pulpitis with different radiographic periapical tissue findings; *$P < 0.05$.

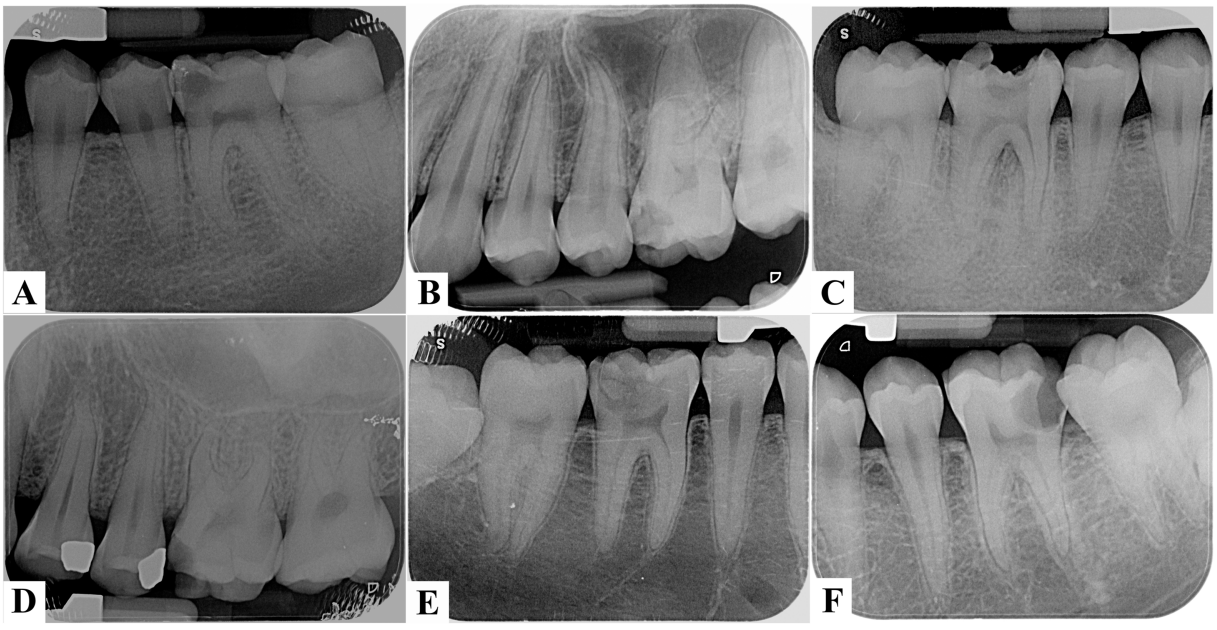

Figure 4 Radiographic findings of periapical tissues in irreversible pulpitis. (A) 36; normal periapical tissues with asymptomatic irreversible pulpitis. (B) 26; widened periodontal ligament space with asymptomatic irreversible pulpitis. (C) 46; periapical lesion with asymptomatic irreversible pulpitis. (D) 26; normal periapical tissues with symptomatic irreversible pulpitis. (E) 36; widened periodontal ligament space with symptomatic irreversible pulpitis. (F) 46; periapical lesion with symptomatic irreversible pulpitis.

surrounding the exposed pulp, the results are consistent with those obtained directly from exposed pulp tissue, suggesting that MMP-8 present in pulpal blood may diffuse into adjacent dentin. This supports the feasibility of using MMP-8 as a clinical biomarker for evaluating the inflammatory status of the pulp.

In alignment with clinical practice, blood samples were collected directly from exposed pulpal wounds rather than from the entire pulp tissue. A substantial number of teeth were excluded due to insufficient blood volume, likely attributed to the use of local anesthesia containing epinephrine, which reduced pulpal blood flow and limited the amount of available blood for analysis. To overcome this limitation, a sterile sharp instrument should be used to induce additional bleeding until an adequate blood volume was obtained. However, this technique introduces the possibility of collecting MMP-8 from mildly inflamed or even healthy tissue, potentially affecting the accuracy of the inflammatory status assessment. Initial attempts to collect blood using pipette tips and collection tubes were unsuccessful, even in cases where visible bleeding appeared adequate, consistent with previous.[17,25] Consequently, a standardized method involving cotton pellets was adopted for blood collection.[14,27]

The sample size for the normal pulp group from premolars which scheduled for extraction for orthodontic reason was limited to five due to the extremely low and consistent levels of MMP-8. To obtain a sufficient volume of pulpal blood, the extraction procedure was temporarily delayed following rubber dam placement and pulp exposure procedure. To minimize patient discomfort, the sample size was kept to a minimum and still sufficient to differentiate among the group. TotalMMP-8 concentration demonstrated higher in irreversible pulpitis teeth with periapical pathology while ActiveMMP-8 concentration showed indifferent statistical significance. This discrepancy may be explained by either the limited sample size for detecting differences in active form of MMP-8 or the involvement of regulatory mechanisms such as tissue inhibitors of metalloproteinases (TIMPs)—which control the activation of MMP-8 and help prevent excessive degradation of pulp tissue.[28]

Future studies should adopt a stepwise approach to pulpal blood sampling that closely mirrors the clinical procedures of VPT. Evaluating MMP-8 concentrations at different depths—such as immediately upon pulpal exposure during caries excavation, after partial and full pulpotomy—may help determine whether MMP-8 levels vary with the extent of tissue removal. In parallel, incorporating additional biomarkers, including inflammatory cytokines (e.g., IL-1β, IL-8, and TNF-α) and neuropeptides such as CGRP and substance P, could guide strategies to remove inflamed pulp tissue until the residual inflammation is minimal enough to promote pulp tissue repair while effectively managing pulpal pain. Complementary histological analyses are also warranted to elucidate the relationship between MMP-8 levels, tissue destruction, and the extent of inflammation.

This study demonstrated that pulpitis teeth exhibited significantly elevated MMP-8 concentrations than normal pulp. However, no statistically significant difference was observed between AIP and SIP. Notably, MMP-8 levels were associated with radiographic periapical pathology. MMP-8 may serve as a promising biomarker for assessing pulpal inflammation.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2025.08.031

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