A Prospective Clinical Pilot Study on the Level of Matrix Metalloproteinase-9 in Dental Pulpal Blood as a Marker for the State of Inflammation in the Pulp Tissue

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Abstract

Introduction: Differentiation between reversible pulpitis (savable pulp) and irreversible inflammation of the pulp tissue (nonsavable pulp) based only on clinical and radiographic diagnoses has proven to be difficult. Pulp exposure allows for the collection of pulpal blood to quantitatively determine the level of inflammation markers or proteolytic enzymes, even with small samples. Pulpitis is associated with the invasion of neutrophil granulocytes and their release of matrix metalloproteinase-9 (MMP-9). **Methods:** Forty-four patients (aged 18–74 years, mean = 35 years), each with 1 tooth with carious pulp exposure presenting with different stages of pulpitis, were included in this prospective, 2-center clinical study; 26 patients presented with irreversible pulpitis (groups 3 and 4), 10 with reversible pulpitis (group 2), and 8 with completely asymptomatic teeth with deep carious lesions (group 1). Six of the 26 patients with teeth diagnosed with irreversible pulpitis had not taken any nonsteroidal anti-inflammatory drugs and were evaluated as a separate group (group 4). Partial pulpotomy and blood sample collection from the pulp chamber were performed. The total levels of MMP-9 and tissue inhibitor of metalloproteinase-1 were assessed by fluorometric and colorimetric enzyme-linked immunosorbent assays, respectively. The Mann-Whitney *U* test and Spearman rank correlations were used to compare the MMP-9 levels with different stages of pulpal inflammation; significance was set at .05. **Results:** The MMP-9 levels in the asymptomatic teeth (group 1) were significantly different from those in the teeth with reversible pulpitis (group 2, $P = .006$) or irreversible pulpitis (group 4, $P < .001$). A statistically significant difference was also observed between the MMP-9 levels in group 1 and group 3 ($P < .001$) in which the patients had taken nonsteroidal anti-inflammatory drugs. **Conclusions:** These findings indicate that the MMP-9 levels in pulpal blood samples could be a useful ancillary diagnostic tool for distinguishing different stages of pulp tissue inflammation. *(J Endod 2016;42:190–197)*

Key Words

Clinical diagnosis, irreversible pulpitis, matrix metalloproteinase-9, pulp tissue inflammation, reversible pulpitis

Dentists are often faced with the problem of deciding on the best therapy after pulp exposure during caries removal. The crucial question is whether the extensive carious lesion has already caused an infection of a large part of the dental pulp resulting in irreversible pulpitis or whether only a small localized part of the pulp tissue close to the carious lesion has been affected. In the first scenario, root canal treatment is indicated (1, 2), whereas in the latter scenario, only a partial pulpotomy (3–5) and dressing of the exposed pulp tissue with a biocompatible material are needed to maintain pulpal vitality and health (6). Therefore, it is important to determine with as much precision as possible whether the inflammatory process is reversible (infection of a localized part of the dental pulp close to the carious lesion) or irreversible (infection of a large part of the dental pulp). Histologic examination of the pulpal tissue would allow an exact differentiation but is only possible after extraction (7–11). A recently published systematic review showed that, to date, none of the clinical parameters that were thought to determine the condition of exposed vital pulps in teeth with different types of damage could be validated by clinical studies (11). Radiographic changes are also rarely noted after irreversible inflammation of the pulp tissue (1). Therefore, distinguishing between the different stages of pulpal inflammation must be effected through clinical diagnostics (12) to decide whether to perform vital pulp therapy (eg, partial pulpotomy) or root canal treatment. The dental "history of pain" and the extent of hemorrhage after pulp exposure also give an indication of the severity of the inflammation (7, 8, 13).

In recent years, matrix metalloproteinases (MMPs), which belong to the family of calcium- and zinc-dependent endopeptidases, have been increasingly studied for their role in the diagnosis of dental inflammatory processes. For instance, an increase in MMP-8 shows a high correlation with periodontal inflammation (14–16) and apical periodontitis (17). Several studies have evaluated the correlation between the inflammation of the dental pulp tissue and the levels of MMPs (MMP-1, -2, -3, -8, and -9) and other molecular markers (interleukins, prostaglandins, and so on) (17–26).

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Of these studies, the results of Gusman et al (24) are of particular interest. This group evaluated the levels of MMP-1, MMP-2, MMP-3, and MMP-9 in clinically healthy and inflamed human dental pulp. The MMP-9 levels of inflamed pulps were significantly higher compared with the clinically healthy pulps. Interestingly, MMP-2 and MMP-3 levels were significantly lower in inflamed pulp compared with healthy dental pulp. The MMP-1 levels were below the detection levels in this study (24).

These results indicate a possible important role of MMP-9 in inflammation processes in dental pulp tissue (27), which is 1 reason MMP-9 was chosen as a marker protein for the present study. The results evaluated by Gusman et al (24) were later supported by other results (25, 26). Tsai et al (25) focused on the examination of tissue obtained after extraction, whereas Zehnder et al (26) analyzed MMP-9 samples from the dentinal fluid of symptomatic human permanent teeth diagnosed with irreversible pulpitis and healthy counterparts.

To date, the use of the concentration of MMP-9 in the pulpal blood as a marker to differentiate between reversible and irreversible pulpitis has not been investigated so there are no reference values showing whether MMP-9 in pulpal blood indicates an irreversible inflammatory process and, if so, in what concentration. In pulpitis, the concomitant expression of the specific inhibitor of MMP-9 activity, the tissue inhibitor of metalloproteinase-1 (TIMP-1), is expected to occur in parallel to MMP-9 release (27). TIMPs reversibly inhibit the proteolytic activity of MMPs (14, 28, 29). Because of this close correlation of MMP-9 and TIMP-1 expression, a relatively high correlation between the MMP-9 and TIMP-1 levels evaluated from the same blood sample is to be expected, at least with regard to the blood samples of pulp with severe inflammation. In addition, a high correlation of all MMP-9 and TIMP-1 levels from the same blood sample could indirectly serve as a control for the accuracy of the measurements performed.

The aim of this prospective, 2-center clinical study was to assess the relationship between different degrees of inflammation in the dental pulp tissue (and thus the clinical diagnoses) and the MMP-9-levels in the pulpal blood. This study also investigated the correlation between the MMP-9 and TIMP-1 values in these blood samples.

Materials and Methods

The study population was composed of patients seeking dental treatment at the Department of Conservative Dentistry of the University Hospital of Heidelberg, Heidelberg, Germany, and patients from a private dental practice located nearby. The treatment provider in the private dental practice has a part-time position as a supervisor in the department. The study protocol of this prospective, 2-center clinical study was approved by the Ethics Committee of the University of Heidelberg (Ref. S-219/2012).

Inclusion Criteria and the Assignment Pulpal Diagnoses

Patients with a tooth showing clinical or radiographic evidence of a deep carious lesion extending close or into the pulp chamber were considered for potential inclusion. All patients who agreed to participate provided their informed consent before treatment and had the right to decline participation in the study at any time.

The teeth were assigned to different groups according to the clinical diagnoses. The diagnoses were based on strict clinical and radiographic criteria. When the dental pulp was exposed during caries removal, the following clinical and radiographic diagnostic criteria were applied:

1. *Group 1* (asymptomatic teeth): No clinical signs or symptoms of pulpitis, no history of pain, response to cold test (with carbon dioxide snow) within normal limits, no sensitivity to percussion or the bite test, bleeding time from the exposed pulp tissue less than 2 minutes, and no widening of the periodontal ligament space (periapical index [PAI] = 1)
2. *Group 2* (teeth with reversible pulpitis): Slight clinical symptoms of minor intensity, slightly exaggerated reaction to cold or sweet stimuli, no history of pain, response to cold test (with carbon dioxide snow) within normal limits, no sensitivity to chewing or percussion, bleeding time from the exposed pulp tissue less than 5 minutes, and no widening of the periodontal ligament space (PAI = 1)
3. *Group 3* (teeth with symptomatic irreversible pulpitis and anti-inflammatory medication [nonsteroidal anti-inflammatory drugs (NSAIDs)]): Patients who used long-acting NSAIDs before treatment. Signs or symptoms could be as follows: history of continuous moderate or severe pain, either provoked or spontaneous; prolonged pain initiated by provocation with carbon dioxide snow; tenderness to chewing or percussion; bleeding time from the exposed pulp tissue longer than 5 minutes; and widening of the periodontal ligament space but no periapical periodontitis (PAI $\leq$ 2)
4. *Group 4* (teeth with symptomatic irreversible pulpitis without NSAID use): Patients did not use long-acting NSAIDs before treatment and clinical or radiographic signs or symptoms identical to those of group 3.

For ethical reasons, there was no control group with blood samples from healthy teeth without deep carious lesions.

Exclusion Criteria

The following exclusion criteria were defined in advance for all groups of teeth: teeth with a negative response to cold test (carbon dioxide snow), apical radiolucency (PAI > 2); condensing apical periodontitis, internal/external root resorption, history of trauma, longitudinal root fracture or evidence of a periodontal-endodontic lesion on the day of treatment, loss of function (eg, tooth mobility grade 3), and swelling in association with the treated tooth. Teeth that could not be treated using rubber dam isolation, teeth from which less than 2.5 μL blood could be obtained from the pulp, and teeth that could not be unequivocally assigned to 1 of the 4 study groups were also excluded. In addition, patients with a compromised immune status; patients who were pregnant at the time of treatment; and patients who had taken antibiotics, bisphosphonates, or statins within the last 4 weeks before treatment were not allowed to participate. For patients in group 3, the use of long-acting anti-inflammatory medications (NSAIDs) in the 14 days preceding treatment was allowed.

Clinical Treatment Intervention and Sample Collection

The flowchart diagram in Figure 1 gives a step-by step overview of the treatment intervention, sample collection, and storage of the blood samples. All treatment interventions were performed under rubber dam isolation. The treatment providers were either dentists or supervised undergraduate students. Caries were removed by means of mechanical excavation with a slow-speed rose head bur. The departmental operating protocol states that the peripheral caries has to be removed before the carious dentin from the cavity walls near the pulp is excavated. If the pulp is exposed, a sterile cotton pellet soaked in sterile physiological saline must be placed on the exposed pulp tissue. At this point, 1 of the clinical investigators responsible for this study temporarily took over the treatment, which involved the verification of complete caries removal, partial pulpotomy, and blood sample collection and afterward the disinfection of the cavity with 0.12% chlorhexidine solution and dressing of the