Metalloproteinases 2 and 9 Immunoexpression in Periapical Lesions from Primary Endodontic Infection: Possible Relationship with the Histopathological Diagnosis and the Presence of Pain

Isabel Schausltz Pereira Faustino, MSc, Rebeca Souza Azevedo, PhD, and Ademar Takahama, Jr, PhD

Abstract

Introduction: The aim of this study was to evaluate the possible associations among the histopathological diagnosis, the inflammatory infiltrate profile, the presence of pain, and the immunoexpression of matrix metalloproteinases MMP-2 and MMP-9 in periapical lesions from primary endodontic infection. **Methods:** Fifty-one primary periapical lesions obtained from extracted teeth were selected for this study. Patients were previously evaluated for the presence of pain and sinus tract related to the tooth to be extracted. Tissues were processed for microscopic examination and MMP-2 and MMP-9 immunoexpression. Microscopically, samples were classified as periapical granulomas or periapical cysts and the inflammatory infiltrate as chronic or mixed. The percentage of immunopositive cells for MMP-2 and MMP-9 of each case was performed based on 10 consecutive microscopic fields. The Student *t* or chi-square tests were used in the statistical analysis. **Results:** Of the total, 28 cases were classified as periapical granulomas (54.90%) and 23 cases as periapical cysts (45.10%). Seventeen patients (33.33%) reported pain associated with the extracted tooth, with 12 cases of periapical granulomas (70.58%) and 5 cases of periapical cysts (29.42%). All cases showed immunopositivity for MMP-2 and MMP-9 in a high percentage of cells, mainly in the cytoplasm of the leukocytes. MMP-2 was expressed more in periapical granulomas than periapical cysts ($P < .05$) and in symptomatic cases ($P < .05$). **Conclusions:** According to the results, we may conclude that MMP-2 and MMP-9 are highly expressed in periapical lesions from a primary endodontic infection. Moreover, we may suggest MMP-2 is expressed more in periapical granuloma and in cases associated with pain. *(J Endod 2016;42:547–551)*

Key Words

Immunohistochemistry, matrix metalloproteinases, pain, periapical granuloma, periapical periodontitis, radicular cyst

Periapical lesions of an endodontic origin represent a periradicular tissue disorder that usually arises from endodontic bacterial infection resulting in local bone resorption (1). They are usually noted as unilocular radiolucencies in the periapical region of a devitalized tooth (2). Microscopically, periapical lesions of endodontic origin are classified as periapical granulomas or periapical cysts (3). Periapical granulomas are composed of a granulation tissue with a variable inflammatory infiltrate surrounded by a fibrous capsule containing fibroblasts, nerve fibers, and newly formed vessels (4). Periapical cysts are known to originate from epithelial rests of Malassez within a periapical granuloma (5). It is lined with a stratified squamous epithelium, which may present exocytosis (6), and a fibrous capsule containing an inflammatory infiltrate similar to a periapical granuloma (4). Periapical lesions of endodontic origin are usually asymptomatic (2). However, Peñarrocha et al (7) determined that approximately 50% of cases are symptomatic.

Matrix metalloproteinases (MMPs) are a family of more than 20 metalloenzymes that cleave components from the extracellular matrix, including interstitial collagen, basement membranes, fibronectin, laminin, and proteoglycans (8). MMP-2 and MMP-9 have been suggested to play an important role in the development of periapical lesions, probably in the degradation of the extracellular matrix (9) with different concentrations when compared with normal tissue (10). MMP-9 may act in the beginning of bone resorption by removing the collagen layer from the bone surface before the demineralization process (11). It has also been shown that MMPs are activated by bacterial proteases (4), such as *Porphyromonas gingivalis* and *Porphyromonas endodontalis*, which can elevate MMP-2 and MMP-9 concentrations (12–14). It is noteworthy that some studies correlated MMPs with the presence of pain in nonvital teeth (15, 16). MMP-2 and MMP-9 may be related to the process of neuropathic pain via proinflammatory mechanisms (17–19). Moreover, they showed different expressions in periapical cysts and periapical granulomas (14). Ahmed et al (16) found a higher MMP-9 expression in symptomatic periapical lesions, suggesting a significant role of this enzyme in these cases.

Thus, the aim of this study was to evaluate the possible associations among the histopathological diagnosis, the inflammatory infiltrate profile, the presence of pain,

From the Universidade Federal Fluminense, Faculdade de Odontologia, Nova Friburgo, Rio de Janeiro, Brazil.

Address requests for reprints to Dr Ademar Takahama Jr, Departamento de Formação Específica, Faculdade de Odontologia, Nova Friburgo, Universidade Federal Fluminense, Rua Doutor Silvio Henrique Braune, 22 Centro CEP: 28625-650, Nova Friburgo, RJ, Brasil. E-mail address: ademartjr@yahoo.com.br

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and the immunoexpression of MMP-2 and MMP-9 in periapical lesions from primary endodontic infection.

Materials and Methods

Sample Selection

Over the course of a year, we selected 51 periapical lesions obtained by curettage during the extraction of permanent teeth that were radiographically diagnosed as presenting periapical lesions with nonvital pulps. Teeth were extracted because there was no possibility of being restored, and obtaining tissue was performed by direct curettage of the dental alveolus. The ethical committee from Universidade Federal Fluminense, Nova Friburgo, Rio de Janeiro, Brazil, reviewed and approved the present study. Patients were examined for the presence of fistula, and teeth were tested for a vital state with a cold thermal test to confirm the pulp necrosis. Patients were then divided into 2 groups: the symptomatic group included patients complaining of pain, and the asymptomatic group included patients without pain. Pain was evaluated by patients' self-report at the moment before surgical procedure. Exclusion criteria were teeth with endodontic treatment, the presence of periodontal disease, and patients using any systemic medication. All surgical procedures were performed under the supervision of an experienced oral surgeon, reducing the chance of an incomplete excision.

Tissue Sample

After tooth extraction, periapical tissues were collected by curettage and immediately immersed in 10% buffered formalin. Upon macroscopic evaluation, the specimens were sectioned to provide more representative areas in the same slide. Samples were processed with an automatic tissue processor (PT09; Lupetec, São Carlos, Brazil) that used sequences with increasing alcohol followed by xylol and finally embedded in paraffin. Blocks were then serially cut into 5-μm sections for the histochemical study and 3-μm sections for immunohistochemical studies.

Microscopic Analysis

Five-micrometer histologic sections were deparaffinized with 2 sequences of xylol, hydrated in decreasing concentrations of alcohol, and washed with tap and distilled water before being kept in hematoxylin for 3 minutes. Histologic sections were differentiated in tap water for 15 minutes and then sequentially immersed in alcohol at 70% and kept in eosin for 5 minutes. Finally, the sections were differentiated in 4 immersions in absolute alcohol and 3 immersions in xylol before being mounted by a coverslip using Entellan (EMD Millipore, Darmstadt, Germany).

Microscopic analysis of the hematoxylin-eosin–stained slides was performed using a Nikon Eclipse E200 optical microscope (Nikon Instruments Inc, Tokyo, Japan). Lesions were classified according to Nair et al (4) in periapical granulomas or periapical cysts. Periapical granulomas were identified by granulation tissue surrounded by fibrous connective tissue even when epithelium was inside (without the presence of cystic cavities), and periapical cysts were diagnosed according to the presence of a cavity lined by epithelium. The inflammatory infiltrate of each case was classified as chronic (the presence of mononuclear leukocytes) or mixed (mononuclear and polimorfonuclear leukocytes).

Immunohistochemistry

Three-micrometer histologic sections were deparaffinized with 2 sequences of xylol, hydrated in ethanol, washed with tap water and then with 10% hydrogen peroxide for 25 minutes to inhibit the action of endogenous peroxidase, and finally washed in tap water again. Antigen retrieval was performed using citrate (pH = 6.0, prepared from citric acid and distilled water) in an electric pressure cooker. After cooling, sections were washed with water and phosphate-buffered saline (PBS) solution. Sections were incubated with the primary mouse monoclonal antibodies MMP-2 (sc-53630, dilution 1:150; Santa Cruz Biotechnology, Santa Cruz, CA) and MMP-9 (sc-21733, dilution 1:500, Santa Cruz Biotechnology) in a humidity chamber for 18 hours at 4°C. Then, sections were washed in PBS, and the secondary antibody conjugated to peroxidase (Strept AB complex/HRP Duet, Mouse/Rabbit; Dako, Carpinteria, CA [1/500]) was then added for 30 minutes at 37°C. Thereafter, sections were again washed in PBS (3 changes) and exposed to a streptavidin-biotin-peroxidase system (Strept Complex AB/HRP Duet, Mouse/Rabbit, Dako, 1:500) for 30 minutes. The chromogen used to reveal the reaction was 3,3′-diaminobenzidine (DAB, Dako) mixed with 200 mL PBS, 2 mL hydrogen peroxide (20 volumes), and 2 mL dimethyl sulfoxide and then incubated in a dry chamber at 37°C for 5 minutes. After this, slides were washed in water, counterstained with Carazzi's hematoxylin, dehydrated in ethanol, diaphonized in xylol, and mounted using a coverslip in Entellan. The positive controls were a liver sample for MMP-2 and a placenta sample for MMP-9, and negative controls were obtained by reactions performed without primary antibodies.

Quantitative Image Analysis

For immunohistochemical evaluation, 10 consecutive fields at 1000× magnification of each case were scanned using the Leica DM750 microscope and Leica Application Suite version 3.0.0 (Leica Microsystems, Heerbrugg, Switzerland). For each field, the number of positive inflammatory cells over the total number of inflammatory cells were counted using Image Pro Plus version 4.5.0.29 for Windows (Media Cybernetics Inc, Sarasota, FL). The percentage of immunopositivity for each case was calculated by the mean of 10 fields. In periapical granulomas, the fields were chosen from the center to the periphery of the lesion and in periapical cysts from the epithelial lining to the capsule.

Statistical Analysis

Data were analyzed using the Student *t*, Mann-Whitney *U*, chi-square, and Pearson correlation tests. *P* values lower than .05 were considered significant. Statistical analysis was performed with Bioestat 5.0 software (Bioestat, Tefé, Brazil).

Results

There was a similar distribution between sex, 53% female and 47% male. The mean age of the patients was 42.12 years, varying from 14–75

TABLE 1. Clinical Features, Inflammation Profile, and Matrix Metalloproteinase (MMP)-2 and -9 Immunoexpression According to the Histopathological Diagnosis

	Periapical granuloma *n* (%)	Periapical cyst *n* (%)	*P* value*
Clinical features			
Sinus tract	3 (10.3)	0 (0)	>.05
Pain	12 (42.85)	5 (21.73)	>.05
Inflammation profile			
Chronic	9 (32.15)	11 (47.82)	<.05
Mixed	19 (67.85)	12 (52.18)	
Immunoexpression			
MMP-2	85.2% ± 0.104†	75% ± 0.124†	<.05
MMP-9	77.3% ± 0.119†	75.4% ± 0.131†	>.05

*Significant = $P < .05$.

†Mean of percentage of positive cells ± standard deviation.