Pulp Inflammation Diagnosis from Clinical to Inflammatory Mediators: A Systematic Review

Marjorie Zanini, DDS, MSc, Elisabeth Meyer, DDS, and Stéphane Simon, DDS, PhD, HDR

Abstract

Introduction: Similar to other tissues, the dental pulp mounts an inflammatory reaction as a way to eliminate pathogens and stimulate repair. Pulp inflammation is prerequisite for dentin pulp complex repair and regeneration; otherwise, chronic disease or pulp necrosis occurs. Evaluation of pulp inflammation severity is necessary to predict the clinical success of maintaining pulp vitality. Clinical limitations to evaluating in situ inflammatory status are well-described. A molecular approach that aids clinical distinction between reversible and irreversible pulpitis could improve the success rate of vital pulp therapy. The aim of this article is to review inflammatory mediator expression in the context of clinical diagnosis. Methods: We searched PubMed and Cochrane databases for articles published between 1970 and December 2016. Only published studies of inflammatory mediator expression related to clinical diagnosis were eligible for inclusion and analysis. Results: Thirty-two articles were analyzed. Two molecular approaches were described by study methods, protein expression analysis and gene expression analysis. Our review indicates that interleukin-8, matrix metalloproteinase 9, tumor necrosis factor-α, and receptor for advanced glycation end products expression increase at both the gene and protein levels during inflammation. Conclusions: Clinical irreversible pulpitis is related to specific levels of inflammatory mediator expression. The difference in expression between reversible and irreversible disease is both quantitative and qualitative. On the basis of our analysis, in situ quantification of inflammatory mediators may aid in the clinical distinction between reversible and irreversible pulpitis. (J Endod 2017;43:1033–1051)

Key Words

Dental pulp, diagnosis, gene expression, inflammation, molecular pattern

Significance

Because of the limitations of accuracy of clinical diagnosis tools, outcomes after vital pulp therapy are unpredictable. Molecular-based diagnostic strategies are promising and may be relevant to determine proper clinical indications for vital pulp therapies and optimize prognosis.

Introduction:
The goal of any restorative or endodontic procedure should be to maintain dental pulp vitality and functionality without patient discomfort. Pulp tissue is necessary for tooth nutrition, innervation, and immunocompetency. Maintaining dental pulp vitality increases tooth mechanical resistance and long-term survival (1, 2). Therefore, pulp vital therapies such as indirect pulp capping, stepwise caries excavation, direct pulp capping, and pulp chamber pulpotomy may be preferable to root canal treatment in some situations. Unfortunately, the success of vital pulp therapy varies greatly, especially for direct pulp capping after carious excavation (3.1%–91.3%) (3, 4). Clinical early failures (within days or weeks) are multifactorial but certainly may be related to improper diagnosis of pulp disease. Indeed, poor pulp status evaluations may result in underestimation of pulp inflammation severity. This oversight can lead to irreversible pulp inflammation and pulp tissue necrosis, which results in spontaneous and persistent pain after therapy.

Proper evaluation of pulp inflammation and choice of appropriate materials before therapy are the keys to improving pulp vital therapy success. Tricalcium silicate–based cements have excellent sealing properties and bioactive properties because they induce good-quality hard tissue barriers (5–7). Accurate diagnosis requires knowledge of pulp inflammation severity and the likelihood of response to endodontic procedures. Intense or long-term pulpal inflammation that is due to unresolved infection or inflammation precludes regeneration (8). Because inflammation aids in pathogen elimination and repair stimulation, control of pulp inflammation severity is necessary for vital pulp therapy success (9, 10).

Currently, pain quality and history and responses to pulp sensitivity tests are the only clinical tools available to evaluate pulp inflammation severity (11, 12). Pulpal diseases are often clinically classified by using the criteria of the American Association of Endodontists (AAE) (13). The terms reversible or irreversible pulpitis simply reflect the intention of the practitioner to keep or remove vital pulp; therapy could be attempted on a tooth with reversible pulpitis, as defined by the absence of spontaneous pain or absence of pain after stimulation. Root canal treatment remains the therapy of choice for irreversible pulpitis because the pulp is too deeply inflamed to expect recovery.

Unfortunately, the AAE clinical categorization has limitations. Histologic observations show no correlation between clinical diagnosis and in situ pulp status (14, 15). Histologic evaluation of tissues clinically diagnosed as irreversible symptomatic pulpitis...
do not show deep inflammation (16), whereas pulp necrosis may occur in asymptomatic or low-grade symptomatic patients whose disease could be wrongly diagnosed as reversibly inflamed. Therefore, pain characterization that is based on clinical tests seems inadequate to reliably differentiate between reversible and irreversible pulp inflammation. Because clinical diagnosis is unreliable, the outcome of vital pulp therapy is unpredictable, and practitioners may completely remove the pulp more often than is necessary to limit postoperative pain and infection.

Pulp inflammation involves several biological processes evaluable at the macroscopic, microscopic, and molecular levels. Macroscopic changes are mainly noticeable at the vascular level (eg, vasodilatation) (17, 18). Increased immune cell numbers are remarkable on microscopic examination (19). Release of multiple inflammatory biomolecules is also observed. The molecular immune response may precede the cellular immune response, suggesting that cytokines and other signaling molecules are synthesized and secreted by dentin pulp complex host cells before immune cell recruitment and activation (20).

The quality and quantity of these mediators are key to inflammatory evolution, especially with respect to the type of tissue immune response generated. Some mediators guide and amplify the inflammatory process. Type 1 cytokines (eg, interferon-γ, interleukin [IL]-2, IL-12, tumor necrosis factor [TNF]-α) orchestrate strong cellular immune responses, particularly intense phagocytic activity. Other mediators are responsible for tissue repair. Type 2 cytokines (eg, IL-10, IL-4) suppress macrophage activation and phagocytosis and stimulate B-cell proliferation and differentiation into plasma cells after resolution of cell-mediated inflammation (21, 22). Furthermore, the carious lesion model describes deeper carious lesions as having greater quantities of inflammatory mediators and more pulp inflammation (20, 23).

Inflammatory mediators orchestrate the inflammatory process inside the pulp tissue. Consequently, the molecular phase precedes the macroscopic and microscopic inflammatory changes; thus, it would be instructive to study their expression inside pulp tissue both qualitatively and quantitatively relative to the severity of the pulpal disease. The aim of this study was to review articles concerning inflammatory molecule expression related to clinical diagnosis to identify key inflammatory mediators and describe their expression profile in inflammatory pulp disease.

**Methods**

We performed a literature review of expression of any inflammatory mediators related to clinical diagnosis to create a list of potential expression patterns. This systematic review was conducted by using Preferred Reporting Items for Systematic reviews and Meta Analyses

![Figure 1. Flow diagram of records.](image-url)
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<td>Cohen et al, 1985 (44)</td>
<td>Normal pulps, n = 20&lt;br&gt;Reversible pulpitis, n = 30&lt;br&gt;Irreversible symptomatic pulpitis, n = 13</td>
<td>Extraction of teeth and extirpation of pulp tissue.</td>
<td>To measure and compare levels of PGE₂ and PGF₂α in painful and asymptomatic human dental pulps.</td>
<td>Radioimmunoassay</td>
<td>Mean value of PGE₂ in reversible pulpitis group is significantly higher than that for normal pulp group (P &lt; .05). Mean value of PGE₂ in irreversible symptomatic pulpitis group is significantly higher than that for normal pulp group and reversible pulpitis group (P &lt; .01). Mean PGF₂α in irreversible symptomatic pulpitis is significantly higher than the 2 other groups (P &lt; .01). PGF₂α values for normal pulp group and reversible pulpitis group were not significantly different (P &gt; .05).</td>
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<td>Proctor et al, 1991 (45)</td>
<td>Normal pulp&lt;br&gt;Irreversible symptomatic pulpitis or necrotic pulp</td>
<td>Pulp tissue extirpation with spoon excavator or sterile barbed broach.</td>
<td>To determine whether CRP levels in pulp could be correlated with histologic disease status of pulp and with systemic blood levels of the protein.</td>
<td>ELISA assay</td>
<td>Only normal versus inflamed pulp contrast was significant (P &lt; .05). Correlation between serum CRP and pulp CRP was not significant (P = .160).</td>
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<td>Hahn and Falkler, 1992 (46)</td>
<td>Normal pulp, n = 15&lt;br&gt;Irreversible symptomatic pulpitis, n = 38</td>
<td>Extirpated pulp dissected into pieces and tissue culture.</td>
<td>To investigate the humoral immune responses of clinically symptomatic pulpitis and normal pulps by allowing antibodies in SF to react with predominant bacteria isolated from deep carious lesions.</td>
<td>Double diffusion in agar assay</td>
<td>Irreversible symptomatic pulpitis group: 73.68% reacted with goat anti-human Y chain serum, and only 1 specimen had detectable IgA as well as IgG in SF. No IgM was detected. Normal pulp group: IgG was detected in 73.33%. Neither IgA nor IgM was detected in SF. No difference of mean antibody levels between irreversible and normal groups of reactivity of SF to all microorganisms except (continued)</td>
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<td>Rauschenberger et al, 1994 (30)</td>
<td>Normal pulp, n = 9</td>
<td>Normal pulp group: extraction of teeth and extirpation of pulp tissue. Irreversible symptomatic pulpitis group: pulp extirpation with spoon excavator</td>
<td>To determine whether a relationship exists between levels of PMN proteases (PMN-E, PMN-CG) and the common inhibitor (A2-M) in healthy and inflamed dental pulps.</td>
<td>Histologic analysis</td>
<td>9 are classified as healthy, 5 are classified as mildly inflamed, 7 are classified as moderately inflamed to severely inflamed. Moderate to severely inflamed pulpal tissues had significantly greater concentrations of PMN-E than healthy or mildly inflamed tissues (P &lt; .05). Mildly inflamed tissues had significantly less PMN-CG concentrations than normal or moderately to severely inflamed pulpal tissues (P &lt; .05). No significant correlation in healthy or mildly inflamed pulpal tissues concerning PMN-E, PMN-CG, A2-M (P = .005). Significant correlation between A2-M and PMN-CG in moderately to severely inflamed pulpal sample group (P &lt; .05).</td>
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<td>Rauschenberger et al, 1997 (47)</td>
<td>Extraction of pulp and extraction of pulp tissue.</td>
<td>To determine whether IL-2 can be detected in normal</td>
<td>Histologic analysis</td>
<td>14 are classified as healthy, 8 are classified as mildly inflamed</td>
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<td>Barkhordar et al, 1999 (48)</td>
<td>Normal pulp, n = 8</td>
<td>Irreversible symptomatic pulpitis, n = 6</td>
<td>Normal pulp group: extraction of teeth and extraction of pulp tissue. Irreversible symptomatic pulpitis group: non precise.</td>
<td>To study the level of IL-6 in inflamed human pulps by using ELISA. ELISA Healthy pulp tissues showed marginal amount of the cytokine. Significant amounts of IL-6 were detected in all inflamed specimens, compared with normal pulp group (P non-precise).</td>
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<td>Huang et al, 1999 (49)</td>
<td>Normal pulp, n = 15</td>
<td>Irreversible symptomatic pulpitis, n = 14</td>
<td>Normal pulp group: extraction of teeth and extraction of pulp tissue. Irreversible symptomatic pulpitis: pulp extirpation with endodontic spoon excavator (coronal pulp) and file (radicular pulp).</td>
<td>To determine levels of IL-8 expression in normal human dental pulps as well as in pulps that show signs of irreversible symptomatic pulpitis. ELISA Normal pulp group: 53% of samples show detectable but low level of IL-8. Irreversible symptomatic pulpitis group: 71% demonstrated detectable and elevated levels of IL-8. Significant difference (P &lt; .05).</td>
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<td>Rodd and Boissonade, 2000 (50)</td>
<td>Normal pulp, n = 19</td>
<td>Moderately carious teeth, n = 22</td>
<td>Extraction of pulp and extraction of pulp tissue.</td>
<td>To investigate expression of SP within human teeth in both health and disease and to seek a correlation between reported pain history and SP expression. Immunocytochemistry Increase of SP expression in pulp horn as follows: in grossly carious teeth &gt; moderately carious teeth &gt; intact teeth (P &lt; .001). Increase of SP expression in subodontoblastic nerve plexus as follows: in grossly carious teeth &gt; moderately carious teeth &gt; intact teeth (P &lt; .05). (continued)</td>
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<td>Awawdeh et al, 2002 (51)</td>
<td>Normal pulp, n = 20 Irreversible symptomatic pulpitis, n = 46</td>
<td>Extraction of teeth and pulp extirpation with barbed broach or Hedström file.</td>
<td>To compare levels of SP, NKA, and CGRP in human dental pulp tissue from painful and non-painful teeth.</td>
<td>Radioimmunoassay</td>
<td>Increase of SP expression in mid-coronal pulp as follows: in grossly carious teeth &gt; moderately carious teeth &gt; intact teeth ((P &lt; .05)). Increase of SP expression in 3 regions in grossly carious teeth. Significant expression of SP in painful teeth compared with asymptomatic teeth ((P &lt; .05)).</td>
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<td>Anderson et al, 2002 (52)</td>
<td>Normal pulp, n = 24 Irreversible symptomatic pulpitis, n = 32 Necrotic pulp, n = 23</td>
<td>Extraction of teeth and extraction of pulp with forceps.</td>
<td>To confirm whether there are significant differences in IL-2 levels within human pulpal tissues diagnosed as normal, irreversibly inflamed, or necrotic by commonly accepted clinical diagnostic testing procedures.</td>
<td>ELISA</td>
<td>No statistical difference between experimental groups. Mean concentrations of SP, NKA, and CGRP were significantly higher in pulp tissue from painful teeth compared with non-painful teeth ((SP, P &lt; .05; CGRP, P &lt; .05; NKA, P &lt; .0001)). Significant association between severity of pain (visual analogue scale) and levels of SP ((P &lt; .05)), NKA ((P &lt; .001)), CGRP ((P &lt; .05)).</td>
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<td>Gusman et al, 2002 (53)</td>
<td>Normal pulp, n = 18 Irreversible symptomatic pulpitis, n = 17</td>
<td>Normal pulp group: extraction of teeth and extraction of pulp. Irreversible symptomatic pulpitis group: pulp extirpation with Hedström files and spoon excavator.</td>
<td>To study levels of certain selected MMPs, their interdependence, and the overall gelatinolytic activity in both clinically healthy and inflamed human dental pulps.</td>
<td>ELISA</td>
<td>MMP-1 level is below detection limit for both clinically healthy and irreversible symptomatic pulpitis group. Levels of MMP-2 and MMP-3 were significantly lower in symptomatic versus clinically healthy pulp ((P &lt; .001)). MMP-9 value was significantly higher in inflamed pulp than in normal pulp ((P &lt; .001)).</td>
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### Shin et al, 2002 (54)

| Normal pulps, \( n = 10 \) | Irreversible symptomatic pulpitis, \( n = 12 \) | Extirpation of pulp tissue (during root canal treatment) | To evaluate tissue levels of MMP-1, MMP-2, and MMP-3, and their distributions in inflamed human dental pulps and periapical lesions. | Gelatinolytic activity Degradation bands were significantly more abundant and intense in irreversible symptomatic pulpitis groups compared with healthy group (\( P < .001 \)).

Shin et al, 2002(54) Normal pulps, \( n = 10 \) Irreversible symptomatic pulpitis, \( n = 12 \) Extirpation of pulp tissue (during root canal treatment). To evaluate tissue levels of MMP-1, MMP-2, and MMP-3, and their distributions in inflamed human dental pulps and periapical lesions. Immunohistochemistry Irreversible symptomatic pulpitis: MMP-1 and MMP-3 were localized predominantly in infiltrating neutrophils and macrophages. Immunoreactivity of MMP-1 and MMP-3 was stronger and more frequent than that of MMP-2. Irreversible asymptomatic pulpitis: MMP-1, MMP-2, and MMP-3 were expressed in chronic inflammatory cells—plasma cells, lymphocytes, and macrophages. ELISA Concentration of MMP-1 in inflamed group than in normal pulp (\( P < .05 \)). Level of MMP-2 was significantly higher in irreversible symptomatic pulpitis than in control (\( P < .05 \)). Levels of MMP-3 were significantly higher in irreversible symptomatic pulpitis than in control group (\( P < .05 \)).

### Pezelj-Ribaric et al, 2002 (55)

| Normal pulp, \( n = 20 \) | Reversible pulpitis, \( n = 20 \) | Extraction of teeth and extraction of pulp tissue. | To determine whether TNF-\( \alpha \) could be detected in normal, symptomatic, and asymptomatic pulpal tissues classified according to clinical symptoms. | ELISA Expression of TNF-\( \alpha \) in all vital pulp samples. Difference in concentrations was statistically significant between irreversible symptomatic and reversible pulpitis samples (\( P = .000 \)).

### Artese et al, 2002 (56)

| Normal pulp, \( n = 25 \) | Irreversible symptomatic pulpitis, \( n = 25 \) | Non-precise | To evaluate immunostaining for VEGF and factor VIII in normal healthy and inflamed dental pulps. | Immunohistochemistry Expression of VEGF was strongly positive in inflammatory infiltrate of irreversible pulpitis. VEGF expression in stromal cells in healthy pulps ranged from 20% to 100% (continued)
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<td>Bowles et al, 2003 (57)</td>
<td>Normal pulp, ( n = 8 )</td>
<td>Pulp exposure and insertion of microdialysis probe into pulp tissue.</td>
<td>To determine ( in vivo ) pulpal levels of immunoreactive SP in human teeth with diagnosis of normal pulp or irreversible symptomatic pulpitis.</td>
<td>Microdialysis</td>
<td>Levels of induced SP collected by microdialysis were much higher in irreversibly inflamed pulp versus normal pulp (( P = .001 )).</td>
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<td>Irreversible pulpitis, ( n = 16 )</td>
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<td>and in irreversible pulpitis ranged from 0% to 100%; the difference was significant (( P = .05 )). Decrease of microvessel density in irreversible symptomatic pulpitis (( P = .001 )).</td>
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<td>Piattelli et al, 2004 (58)</td>
<td>Normal pulp, ( n = 23 )</td>
<td>Extraction of teeth and extraction of pulp tissue with excavator.</td>
<td>To evaluate and compare TGF-( \beta )1 expression in healthy pulps and in those with irreversible pulpitis.</td>
<td>Immunohistochemistry</td>
<td>Healthy pulps: In 5 cases, more than 50 cells were positive to TGF-( \beta )1 in odontoblastic-subodontoblastic layer. In 6 cases, positive cells were between 10 and 50 cells, whereas 12 cases were negative. Irreversible symptomatic pulpitis: In 15 cases, more than 50 cells were positive to TGF-( \beta )1 in odontoblastic-subodontoblastic layer. In 2 cases, positive cells were between 10 and 50 cells, whereas 3 cases were negative. Higher expression of TGF-( \beta )1 was found in odontoblastic-subodontoblastic layer of irreversible pulpitis, and this difference is statistically significant (( P = .0002 )).</td>
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<td>Irreversible symptomatic pulpitis, ( n = 20 )</td>
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<td>Caviedes-Bucheli et al, 2004 (59)</td>
<td>Normal pulp, ( n = 5 )</td>
<td>Normal pulp: extraction of teeth and extraction of pulp. Irreversible symptomatic pulpitis and induced pulp inflammation groups: pulp extirpation with sterile barbed broach.</td>
<td>To determine tissue levels of CGRP in human pulpal samples collected from teeth with clinical diagnosis of acute irreversible pulpitis, in teeth with induced inflammation, and in teeth</td>
<td>Flow cytometry</td>
<td>Significant statistical differences were found between percentages of CGRP expression in healthy pulps and pulps with acute irreversible pulpitis (( P &lt; .005 )). No difference between induced inflammation</td>
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<td>Irreversible symptomatic pulpitis, ( n = 5 )</td>
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<td>Induced pulp inflammation, ( n = 5 )</td>
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<td>Nakanishi et al, 2005 (60)</td>
<td>Normal pulp, n = 5</td>
<td>Extraction of teeth and extraction of pulp with explorer and/or spoon excavator.</td>
<td>To study MIP-3α and CCR6 expressing cells in normal and inflamed dental pulp tissue and to elucidate whether MIP-3α contributes to pathogenesis of pulpitis.</td>
<td>Immunohistochemistry: Normal pulp group: MIP-3α was detectable, whereas CCR6 was not detectable. Irreversible symptomatic pulpitis group: All inflamed pulp contained MIP-3α–producing and CCR6–positive cells. MIP-3α was detected in CD68-positive monocytes/macrophages adjacent to carious lesions. Few microvascular endothelial cells located near the bacterial invasion displayed MIP-3α immunoreactivity. CCR6 expression was observed in infiltrating lymphocytes.</td>
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<td>Tsai et al, 2005 (73)</td>
<td>Normal pulp, n = 14</td>
<td>Extraction of teeth and extraction of pulp tissue with spoon excavator.</td>
<td>To study levels of MMP-9 in both clinically healthy and inflamed human dental pulps.</td>
<td>Immunohistochemistry: MMP-9 staining was detected in clinically healthy pulps. MMP-9 staining was stronger in inflamed pulps than in clinically healthy pulps. MMP-9 was observed mainly in cytoplasm of odontoblasts, fibroblasts, inflammatory cells, and endothelial cells in inflamed pulps. Significantly greater MMP-9 expression was noted in inflamed human dental pulps than those of clinically healthy pulps (P &lt; .02).</td>
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<td>Caviedes-Bucheli et al, 2006 (61)</td>
<td>Normal pulp, n = 6, Irreversible symptomatic pulpitis, n = 6</td>
<td>Normal pulp: extraction of teeth and extraction of pulp. Irreversible symptomatic pulpitis and induced pulp inflammation groups: pulp extirpation with sterile barbed broach.</td>
<td>To study these neuropeptides and understand their role in pulp neurophysiology.</td>
<td>Radioimmunoanalysis: 5 neuropeptides (SP, CGRP, NKA, NPY, VIP) were identified in all samples. Significantly higher expression of neuropeptides in pulps with irreversible symptomatic pulpitis (P &lt; .0001), except for VIP, which is unaltered in the 3 groups (P &gt; .05).</td>
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<tr>
<td>Caviedes-Bucheli et al, 2007 (62)</td>
<td>Normal pulp, n = 5, Irreversible symptomatic pulpitis, n = 5</td>
<td>Normal pulp: extraction of teeth and extraction of pulp. Irreversible symptomatic pulpitis and induced pulp inflammation groups: pulp extirpation with sterile barbed broach.</td>
<td>To compare SP receptor expression in human pulp</td>
<td>Radioreceptor assay: SP receptor expression was found in all samples. (continued)</td>
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<td>Sattari et al, 2009 (63)</td>
<td>Normal pulp, n = 16</td>
<td>Normal pulp group: extraction of teeth and extraction of pulp.</td>
<td>To evaluate possible relationship between pulp polyp formation and allergy.</td>
<td>ELISA</td>
<td>IgE was found in 100% of pulp polyps and 50% of normal pulps. Histamine was found in 100% of pulp polyps and 12.5% of normal pulps; the difference is significant (P &lt; .001). Significant positive correlation was found between concentration of histamine and pulp polyp formation (P &lt; .001). IL-4 was found in 62.5% of pulp polyps and 18.75% of normal pulps. The difference is significant (P &lt; .01). IL-12 was found in 25% of pulp polyps and 12.5% of normal pulps (the difference is not statistically significant). Normal pulp group: Presented negative immunolabeling for IL-1β and IL-8 antibodies. Reversible pulpitis group: Presented intense staining specific for both antibodies and also specific and restricted to inflammatory cells. Statistically significant difference was found between normal pulp group and reversible symptomatic pulpitis group (P &lt; .005). Statistical difference was found between induced inflammation group and irreversible symptomatic pulpitis group (P &lt; .05).</td>
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<td>Irreversible asymptomatic pulpitis (pulp polyp), n = 16</td>
<td>Irreversible asymptomatic pulpitis group: extirpation of pulp with sterile excavator.</td>
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<td>Silva et al, 2009 (64)</td>
<td>Normal pulp, n = 10</td>
<td>Normal pulp: extraction of teeth and extraction of pulp.</td>
<td>To analyze presence, location, distribution, and concentration of these cytokines in healthy and inflamed dental pulps by immunohistochemistry and ELISA.</td>
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<td>Reversible pulpitis, n = 10</td>
<td>Reversible pulpitis group: during pulpectomy procedure.</td>
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<td>Immunohistochemistry and ELISA</td>
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Kangarlou Haghighi et al, 2010 (66)

**Normal pulp, n = 20**

**Reversible pulpitis, n = 20**

Extraction of teeth and pulp extraction.

To determine relation between pulpal neuropeptides (SP and CGRP) and caries.

**ELISA**

CGRP was found in 5% and 20% of intact and carious samples, respectively.

SP was present in 40% and 60% of intact and carious samples, respectively.

SP level is significantly increased in reversible pulpitis group compared with normal pulp group ($P < .05$).

Zehnder et al, 2011 (65)

**Normal pulp, n = 12**

**Irreversible symptomatic pulpitis, n = 19**

Dentinal fluid collection

To determine whether MMP-9 levels in dentinal fluid were detectable and differed between pulps from symptomatic teeth diagnosed with irreversible pulpitis and healthy counterparts.

**MMP-9 assay**

Normal pulp group: No detectable level of MMP-9 was found.

Irreversible symptomatic pulpitis group: detectable levels of MMP-9 were found in 7 samples.

Level of MMP-9 was significantly higher in irreversible symptomatic pulpitis group ($P < .05$).

Abd-Elmeguid et al, 2012 (67)

**Normal pulp, n = 5**

**Reversible and irreversible pulpitis, n = 40**

Extraction of teeth and pulp extraction.

To examine presence of DMP-1 in inflamed pulps and understand its role in dental pulp inflammation.

**Immunohistochemistry**

Normal pulp group: DMP-1 was not expressed in mature teeth.

Inflamed pulp group: DMP-1 was present in all samples and localized in both subodontoblastic cell layer in the periphery and the center of the pulp tissue. Intense DMP-1 staining in areas of fibrosis and calcification was found.

Accorsi-Mendonça et al, 2013 (68)

**Normal pulp, n = 10**

**Irreversible symptomatic pulpitis, n = 10**

Normal pulp group: extraction of teeth and extraction of pulp. Irreversible symptomatic pulpitis group: extirpation of pulp during root canal treatment.

To investigate gelatinolytic activity of MMP-2 and MMP-9, TIMP-2, MPO expression in healthy and inflamed dental pulps.

**ELISA**

Statistically significant difference in levels of TIMP-2 was found between healthy and inflamed groups ($P < .005$).

Higher diversity of band degradation was observed in inflamed pulp when compared with healthy group.

Degradation bands of total MMP-2 were significantly more intense in inflamed group ($P = .038$).

**Zymography**

Higher amounts of MPO concentrations were found in inflamed group ($P = .038$).

**MPO assay**

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<th>Objective of the study</th>
<th>Methods</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abd-Elmeguid et al, 2013 (69)</td>
<td>Normal pulp, $n=30$ Reversible pulpitis, $n=23$ Irreversible symptomatic pulpitis, $n=12$</td>
<td>Extraction and pulp extirpation.</td>
<td>To localize OCN in inflamed pulps, to distinguish different levels of OCN in 2 stages of pulp inflammation.</td>
<td>Multiplex assay</td>
<td>OCN expression in reversible pulpitis is significantly higher than in irreversible pulpitis, and both were significantly higher than in controls. OCN expression in reversible pulpitis was positively correlated to expression of VEGF, FGF, MIP-1β, monocyte-derived chemokine, monocyte chemoattractant protein-1, IL-17, and soluble IL-2 receptor α and negatively correlated with that of IL-1α, IL-1β, IL-8, GM-CSF, and MIP-1 α. Normal teeth: No evidence of OCN expression was found. Reversible and irreversible pulpitis tissues had higher OCN levels compared with control tissues. OCN levels were higher in reversible pulpitis compared with irreversible pulpitis. OCN in inflamed tissues was localized in cells and matrix around calcification areas and in cells around blood vessels.</td>
</tr>
<tr>
<td>Tancharoen et al, 2014 (74)</td>
<td>Normal pulp, $n=15$ Irreversible symptomatic pulpitis, $n=15$</td>
<td>Extraction of teeth pulp tissue was removed by using sterile dental probe and forceps.</td>
<td>To investigate expression of RAGE in human dental pulpitis.</td>
<td>Immunohistochemistry</td>
<td>Immunohistochemistry</td>
</tr>
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</table>
RAGE expression in infiltrating inflammatory cells in interstitial connective tissues was intensely stained. Western blot

RAGE protein quantity, normalized to β-actin, showed significant differences in RAGE expression in pulpitis group compared with normal pulp group ($P < .001$).

Mente et al, 2016 (70) Normal pulp, $n = 8$
Reversible pulpitis, $n = 11$
Irreversible symptomatic pulpitis and anti-inflammatory drugs (NSAID), $n = 20$
Irreversible symptomatic pulpitis and absence of anti-inflammatory drugs (NSAID), $n = 6$

Blood sample from exposed pulp by using microcapillary tube.

To determine level of inflammation markers of proteolytic enzymes.

MMP-9 and TIMP-1 assay

MMP-9 levels in normal pulp group were significantly different from those in teeth with reversible pulpitis or irreversible pulpitis ($P = .006$, $P < .001$, $P < .001$, respectively).

Statistically significant difference was observed between MMP-9 levels in reversible pulpitis group and irreversible symptomatic pulpitis group (without NSAID) ($P = .005$).

MMP-9 and TIMP-1 levels showed very highly significant correlation regardless of group assignment with positive correlation coefficient ($P < .001$).

A2-M, α2 macroglobulin; CCR6, chemokine receptor 6; DMP, dentin matrix protein; FGF, fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; Ig, immunoglobulin; MIP, macrophage inflammatory protein; MPO, myeloperoxidase; NSAID, nonsteroidal anti-inflammatory drug; OCN, osteocalcin; PMN-CG, PMN cathepsin G; PMN-E, PMN elastase; SF, supernatant fluid; VIP, vasoactive intestinal peptide.
**Selection Criteria**
Articles that met the following criteria were included in the final selection: articles that concern human permanent mature teeth, articles with a clear clinical diagnosis of inflammatory pulp disease (in accordance with the AAE), studies that included a search for inflammatory mediators, and articles that were published in English.

**Literature Search**
The electronic databases PubMed and Cochrane were searched by using a key search terms combination relevant to the research topic (Fig. 1): [PULP TISSUE] AND [GENE OR PROTEIN EXPRESSION] AND [DIAGNOSIS]. Articles published between 1970 and December 2016 were included in this screening process. We also considered grey literature according to recommendations from Yaylali and阿拉格 (25). In addition, to increase the exhaustivity of the research, the reference lists of selected articles were screened to find studies that might qualify for this review (backward research). This search was made by 2 independent investigators (M.Z., S.S.).

**Data Extraction and Analysis**
A first group of 22 publications was selected, and backward research produced 79 additional articles, 29 of which were eligible. Full texts of qualified articles were then read and selected. Among the 51 full-text articles assessed for eligibility, exclusion criteria were applied to disqualify the following articles: an article reporting a technical development (26), studies in which the clinical diagnosis was not described (27–37), in vitro study (38), cell culture studies (39, 40), a study with immature teeth (41), studies in which the comparison with a disease state was not made (29, 39, 42), and a study in which only macroscopic and microscopic changes had been investigated (43).

**Results**
This review included 32 articles (Tables 1 and 2). The analysis showed that authors used methods of messenger RNA or protein quantification of inflammatory mediators during inflammation; 28 addressed protein expression profiles (30, 44–70), 2 addressed gene expression profiles (71, 72), and 2 addressed both molecular and gene expression (73, 74).

**Sampling Methods**

**Method Variability**
Various methods were used to evaluate protein and gene expression: enzyme-linked immunosorbent assay (ELISA) (30, 45–49, 52–55, 63–66, 68, 70), multiplex assay (69), radioimmunoassay (44, 51, 61, 62), immunohistochemistry (49, 54, 56, 58, 60, 64, 67, 69, 73, 74), microdialysis (57), zymography (68), immunocytochemistry (50, 59), Western blot (74), and reverse transcriptase polymerase chain reaction (RT-PCR) (71–74). The ELISA test was the most used technique in the selected articles.

**Discussion**
The main limitation of this study is the lack of quality studies. Furthermore, the variability in study methods and sample sizes are secondary limitations that preclude meta-analysis. The results of this systematic review suggest that the gene and protein expression of IL-8, TNF-α, MMP-9, and RAGE increase inside pulp samples of teeth clinically diagnosed with irreversible symptomatic pulps.

IL-8 is produced by several pulpal cells including macrophages, lymphocytes, fibroblasts, and endothelial cells (75). In *in vitro* conditions, odontoblast cells exhibit low level IL-8 expression that significantly increases with pathogen-associated molecular pattern stimulation, especially lipopolysaccharide. This increased IL-8 expression is correlated with increased polymorphonuclear neutrophils (PMNs) within the pulp (49) because IL-8 induces neutrophil chemotaxis and release of degradation enzymes during degradation. Thus, IL-8 is often described as the primary regulatory molecule in the acute inflammatory response, and elevated levels may perpetuate and exacerbate the acute inflammatory response. This activity may explain the review findings of increased IL-8 expression in pulp tissue with irreversible pulps. Furthermore, human fibroblast nemosis is the process of cell activation and death that generates inflammatory mediators such as PGs and growth factors (76). Reported increased secretary IL-8 expression in human dental pulp fibroblast (HDPF) spheroids suggests that the HDPF nemosis response promotes the secretion of chemokines such as IL-8 in *in vitro*. Fibroblast nemosis has gained attention because of its potent role in pulp repair. HDPF spheroids have been shown to promote human dental pulp stem cell migration (77); however, further studies are required to elucidate the role of IL-8 in nemosis and pulp regeneration.

TNF-α is a pleiotropic molecule that increases leukocyte toxicity, stimulates acute phase inflammatory proteins, and induces inflammatory cytokine production. TNF-α is an anti-inflammatory mediator essential to the dental pulp response to infection (55, 64). Its synthesis by macrophages is often stimulated by toll-like receptor ligand binding in the presence of bacteria. Because inflammation is a prerequisite to regeneration, certain inflammatory mediators have a role in both inflammation and regeneration. Both roles are concentration-dependent and time- or context-dependent. Interestingly, TNF-α synthesis may be induced directly in response to extraction of matrix proteins.

**Variability of the Molecular Types**

Table 3 lists all the types of molecules investigated in the included studies of this review.

Prostaglandins (PGE₂, PGF₂α), C-reactive protein (CRP), IL-6, substance P (SP), calcitonin-related gene peptide (CGRP), neurokinin A (NKA), neuropeptide Y (NPY), transforming growth factor (TGF)-β1, and vascular endothelial growth factor (VEGF) were studied at the protein level to compare teeth clinically diagnosed with irreversible symptomatic pulps with teeth with normal pulp.

Increased matrix metalloproteinase (MMP)-9, TNF-α, IL-8, and receptor for advanced glycation end products (RAGE) expression was evident at both gene and protein levels for teeth clinically diagnosed with irreversible pulps compared with teeth with normal pulp. Because RAGE has been investigated only once in the context of the research question, further studies should be conducted to determine the reliability of this inflammatory mediator. Contradictory results for MMP-2 and MMP-3 prevent us from drawing conclusions regarding these mediators (53, 54); further studies are required.

Expression patterns of MMP-9, TNF-α, RAGE, and IL-8 are synthesized in Figure 2.
### TABLE 2. Selected Studies Analyzing Gene Expression of Inflammatory Mediators

<table>
<thead>
<tr>
<th>Authors, date (references)</th>
<th>Clinical diagnosis, sample size</th>
<th>Sample collection</th>
<th>Objective of the study</th>
<th>Methods</th>
<th>Results</th>
</tr>
</thead>
</table>
| Zehnder et al, 2003 (71)   | Normal pulp, n = 13
Irreversible symptomatic pulpitis, n = 11 | Normal pulp group: extraction of teeth and collection of pulp with excavator. Irreversible symptomatic pulpitis: extirpation of pulp with excavator and Hedström file. | To investigate differences in cytokines gene expression between pulps from healthy teeth and from symptomatic vital teeth with severe caries lesions. | RT-PCR | Significantly higher mRNA levels for IL-6 \(P < .05\) and especially IL-8 and IL-18 in symptomatic versus healthy pulps \(P < .005\). Contents of IL-1α and IL-1β mRNA between 2 groups scantily failed to reach statistical significance \(P = .06\) and .26, respectively. Significant correlations were observed between IL-1α and IL-1β, between IL-6 and IL-18, and between IL-8 and IL-18 \(P < .05\). |
| Tsai et al, 2005 (73)      | Normal pulp, n = 14
Irreversible symptomatic pulpitis, n = 14 | Extraction of teeth and extraction of pulp tissue with spoon excavator. | To study levels of MMP-9 in both clinically healthy and inflamed human dental pulps. | RT-PCR | Significantly greater MMP-9 expression was noted in inflamed human dental pulps \(>2.1\) than those of clinically healthy pulps \(P < .05\). |
| Kokkas et al, 2007 (72)   | Normal pulp, n = 6
Reversible pulpitis, n = 6
Irreversible symptomatic pulpitis, n = 6 | Normal pulp group: extraction of teeth and extirpation of pulp with spoon excavator OR extraction of pulp with barbed broach. Reversible and irreversible symptomatic pulpitis groups: extirpation of pulp with barbed broach. | To evaluate whether correlation between TNF-α gene expression in human dental pulp and clinically defined reversibility of pulp inflammation could be detected. | RT-PCR | TNF-α expression detected in all 3 groups. Statistical difference in irreversible symptomatic group compared with either normal pulp group \(P = .002\) or reversible pulpitis group \(P = .015\). No statistical difference between normal pulp group and reversible pulpitis group \(P = .7\). |
| Tancharoen et al, 2014 (74)| Normal pulp, n = 15
Irreversible symptomatic pulpitis, n = 15 | Extraction of teeth and pulp tissue was removed by using sterile dental probe and forceps. | To investigate expression of RAGE in human dental pulpitis. | RT-PCR | Overexpression of RAGE mRNA in pulpitis samples \(P < .001\). |
released by bacterial demineralization (78). TNF-α can activate the p38 mitogen-activated protein kinase pathway and induce odontoblast-like cell differentiation into dental pulp stem cells by increasing dentin sialoprotein and dentin phosphoprotein expression and forming tertiary dentin (79). High TNF-α concentrations downregulate alkaline phosphatase, osteopontin, osteocalcin, osterix, and Runx2 expression in dental pulp stem cells, whereas low TNF-α concentrations produce the opposite biological effects (80). Thus, high TNF-α concentrations inhibit pulpal cell mineralization through the Wnt/β-catenin pathway. These findings have been confirmed in mesenchymal stem cells (81) and human apical papilla cells (82). On the basis of our review, pulp tissues clinically diagnosed with irreversible pulpitis show increased TNF-α status at which regeneration is impossible. TNF-α and human apical papilla cells (82). On the basis of our review, pulp tissues clinically diagnosed with irreversible pulpitis show increased TNF-α status at which regeneration is impossible. TNF-α and human apical papilla cells (82). On the basis of our review, pulp tissues clinically diagnosed with irreversible pulpitis show increased TNF-α status at which regeneration is impossible. TNF-α promotes apical papilla cell mineralization in short-term cultures but inhibits mineralization in long-term cultures (82). Interestingly, short-term exposure of cells to TNF-α (6 and 12 hours) induces apoptosis with VEGF upregulation and nuclear factor kappa B signaling, whereas long-term (chronic) exposure (14 days) increases proliferation and shortens the telomere (83). TNF-α triggers neutrophils to release large amounts of reactive oxygen intermediates and promotes neutrophil degranulation (84–86). TNF-α also plays a crucial role in neutrophil survival, particularly through induction of apoptosis; however, the mechanisms underlying this activity have not been defined (87).

MMPs are a family of 21 zinc-dependent endopeptidases that are responsible for structural extracellular matrix (ECM) protein degradation (88). MMPs are essential to tissue homeostasis and play a role in pathologic conditions, especially pulpal inflammation. MMPs alter cellular adhesive properties to aid in cellular migration through the ECM. Their role in tissue destruction is evident but not well-understood. Pulp tissue destruction is partially regulated by MMPs and tissue inhibitors of MMPs (TIMPs) (88). MMP family proteins have dual roles in inflammation pathogenesis, stimulation of protective innate and adaptive immune functions, and tissue destruction (89). Some MMPs are involved in pulp repair. MMP-3 enhances proliferation, migration, and survival of human umbilical vein endothelial cells in vitro (90). Furthermore, topical application of MMP to injured pulp tissues of rat incisors accelerates tissue regeneration, angiogenesis, and reparative dentin formation (90). MMP-3 produces anti-inflammatory effects by decreasing the number of macrophages and antigen presenting cells and significantly inhibiting IL-6 expression (91). MMP-9 or gelatinase (a collagenase family member) is secreted primarily by PMNs, which are abundant in inflamed tissues compared with healthy tissues. Because their main role is to degrade collagen extracellular ground substance, MMP-9s are usually considered as markers of pulp tissue breakdown (92). The role of MMPs in pulpal disease is supported by periodontal studies of MMP levels (especially MMP-9) in gingival crevicular fluid (93–96). MMP-9 levels increase with severity of the periodontal destruction such that MMP-9 could serve as a marker of connective tissue breakdown (96).

There is evidence for IL-8, TNF-α, and MMP-9 linkage, which is based on findings that TNF-α and IL-8 can induce rapid MMP-9zymogen release in whole human blood (97). Furthermore, these 3 biomolecules are associated with neutrophils; IL-8 induces neutrophil chemotaxis, TNF-α is a powerful priming neutrophil agonist, and MMP-9 is secreted primarily by PMNs. Pulpitis is clearly a PMN-driven inflammation; it usually starts with neutrophil invasion into the pulp adjacent to infected dentin. Neutrophils actively defend against pulp infection; however, they can also induce irreversible tissue damage. Neutrophils release MMP to recruit immune cells to the pulp and release reactive oxygen intermediates, including superoxide anions, hydrogen peroxide, hydroxyl radicals, and other potent oxidants, which may cause further tissue destruction.

### Table 3. Molecule Types in the Included Studies

<table>
<thead>
<tr>
<th>Molecule types References</th>
<th>Prostaglandins (P) (44)</th>
<th>C-reactive protein (CRP) (45)</th>
<th>Immunoglobulins (Ig) (46, 63)</th>
<th>Interleukins (IL)</th>
<th>Neurogenic mediators (NPY) (61)</th>
<th>Matrix metalloproteinase (MMP) (53, 54, 68)</th>
<th>Cytokines (55, 69, 72)</th>
<th>Growth factors (56, 69)</th>
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<tbody>
<tr>
<td>IL-1α and IL-1β</td>
<td></td>
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<td></td>
<td>IL-2</td>
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<td>MMMP-2</td>
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<td>Vascular endothelial growth factor (VEGF)</td>
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<td>TNF-α</td>
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<td>IL-8</td>
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<td>MMMP-3</td>
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<td>Fibroblast growth factor (FGF)</td>
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<td>IL-1β</td>
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<td>IL-18</td>
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<td>MMP-9</td>
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<td>Platelet-derived growth factor (PDGF)</td>
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<td>MIP-1α</td>
<td></td>
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<td>Interferon-γ</td>
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<td>Macrophage inflammatory protein 1 receptor (MIP-1α)</td>
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<td>MIP-3α</td>
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<td>Neurokinin A (NKA)</td>
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<td>Transforming growth factor-β (TGF-β)</td>
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<td>Neuropeptide Y (NPY)</td>
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<td>Vascular endothelial growth factor (VEGF)</td>
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<td>Tumor necrosis factor (TNF-α)</td>
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<td>Fibroblast growth factor (FGF)</td>
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<td>Macrophage inflammatory protein 1 receptor (MIP-1α)</td>
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<td>Neurokinin A (NKA)</td>
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<td>Transforming growth factor-β (TGF-β)</td>
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<td>Neuropeptide Y (NPY)</td>
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<td>Receptor for advanced glycation end products (RAGE)</td>
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<td>Tumor necrosis factor (TNF-α)</td>
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<td>Cathepsin G</td>
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<td>Macrophage inflammatory protein 1 receptor (MIP-1α)</td>
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<td>Osteoclast</td>
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<td>Neurokinin A (NKA)</td>
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<td>Dentin matrix protein 1 (DMP-1)</td>
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Figure 2. Expression patterns of MMP-9, TNF-α, RAGE, and IL-8 according to the clinical diagnosis.
enzymes. Gradual tissue necrosis is thus initiated by microabscess formation, which then leads to pulp necrosis (98, 99). By extrapolation, high IL-8 and MMP-9 levels indicate increased neutrophil levels and could be considered markers of tissue breakdown.

Molecular diagnosis may be defined as techniques analyzing both the genome and proteome by applying molecular biology to medical testing. These strategies have revolutionized clinical medicine, particularly in the fields of infectious diseases, oncology, pharmacogenomics, and prenatal genetic testing. Testing is based on expressions of biomarkers, which have been used objectively as indicators of physiologic health, pathogenic processes, and pharmacologic responses to therapy.

The first step toward molecular diagnosis of dental pulp disease is identification of key inflammatory mediators and increased understanding of their role in inflammation and regeneration. Because of the complicated nature of inflammatory pulp disease, it is unlikely that a single mediator will provide sufficient information to diagnose irreversible inflammatory disease. The second step is to determine cutoff points for tests using these inflammatory mediators. Cutoff points will be based on levels of pulp tissue inflammatory mediators judged to reflect the limits of reversible pulp tissue disease. The third step is to develop clinical tools and methods to detect these biomolecules with qualitative and quantitative accuracy. The methods should be feasible in clinical practice and executable chairside to allow for their adoption into therapy. On the basis of findings from this systematic review, evaluation of protein expression appears to be the most appropriate method for clinical evaluation of dental pulp inflammation. Compared with other immunoassay methods, ELISA appears to be the most accurate, sensitive, and specific test for molecular evaluation of dental pulp inflammation. According to the review, 2 types of sampling are suggested, dentinal fluid sampling and pulp blood analysis. The dentinal fluid may serve as a liquid biopsy to assess the state of the underlying pulp by determining component concentrations of the pulp tissue fluid (100). Dentinal fluid is easily collected and considered a serum-derived tissue fluid containing serum proteins and immunoglobulins (the protein concentration is about one fifth that of plasma). Thus, dentinal fluid may serve as a “patient specific diagnostic test for pulp disease” (100, 101). This method is theoretically promising because it is noninvasive. However, the difficulty of collecting sufficient dentinal fluid makes its use clinically impractical (101, 102). Blood evaluation at the site of pulp exposure has been tested (57, 70, 71), but its main disadvantage is its invasiveness. However, pulp blood collection using heparinized microcapillary tubes or microdialysis has proven practical and reliable (70).

Conclusion and Perspectives

Because dental pain descriptions are subjective, clinical diagnosis alone is unreliable to accurately determine pulp inflammation severity and pulp tissue ability to respond to endodontic procedures. As a result, pulp vital therapy is usually performed following empirical decision-making criteria. The need for new pulp diagnostic tools is increasingly evident. Molecular-based strategies are promising and may be relevant to determine appropriate clinical indications for vital pulp therapy and to optimize prognosis after pulp vitality therapy.

By providing more precise diagnoses, molecular-based strategies will change the decision-making of dental practitioners and enhance patient assessment and treatment toward customized therapies. Decreasing root canal overtreatment provides both biological and socioeconomic advantages for patients.

Acknowledgments

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