

## The Effect of Intracanal Medicament Using Calcium Hydroxide in Root Canal Infection Toward MMP-9, TIMP-1 and EGF Level in Apical Lesion: a Literature Review

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### Abstract

Root canal infection is one of periodontitis spectrum that started by dental caries process. There are many bacteria causing root canal infections, mainly obligate anaerobe bacteria. Root canal infection can form apical lesions resulted from the interaction between bacteria and inflammatory cells. The apical lesion can be causing degradation of the extracellular matrix in the root canal system. Collagen degradation is the main characteristic of the apical lesion, which degradation process mediated by pro-inflammatory cells. One of them is a matrix metalloproteinase (MMP) mainly MMP Type-9 (MMP-9). Besides MMP, tissue inhibitor of MMP (TIMP) and epidermal growth factor (EGF) also play a role as MMP regulators. Root canal treatment with intracanal medicament needed to fix dental tissue destruction. There are many intracanal medicaments available, which is calcium hydroxide mostly used in dental therapy. Calcium hydroxide has an antibacterial effect and can be lowering inflammatory cells number. In apical lesion, immune-inflammatory response happened which inflammatory cells play an important role in extracellular matrix degradation. Studies showed that intracanal medicament using calcium hydroxide proven lowering inflammatory cells levels such as MMP-9, TIMP-1 and EGF. This review will have further discussion about calcium hydroxide mechanism in reducing MMP-9, TIMP, and EGF levels in the apical lesion.

**Keywords:** *Root canal infection, Intracanal medicament, MMP-9, TIMP-1, EGF.*

### Introduction

Root canal infection is known as one of periodontitis disease which inflammation process occurs in the apical part.<sup>1</sup> Root canal infection mostly was seen in a patient with neglected dental caries. Root canal infection can form apical lesion which caused by soft tissue and hard tissue destruction in apical area.<sup>2</sup> Epidemiological data showed apical periodontitis happened in 50% population age 50 years and 62% in population age more than 60 years with quite high prevalence in worldwide.<sup>3</sup> Data from Indonesia Health Ministry in 2010 showed that in 2009 pulp and periapical disease occupy as rank eight from 10 diseases with the most significant outpatient number in Indonesia hospitals.<sup>4</sup> To overcome root canal infection, endodontic treatment is needed to kill all of the bacteria

in the root canal system. Irrigation and canal system treatment mechanically are essential steps to decrease the number of bacteria in endodontic treatment.<sup>5,6</sup> Intracanal medicament helps to eliminate bacteria that are resistant to mechanical chemical preparation and prevent reinfection in the root canal system between visits.<sup>6,7</sup>

In root canal infection with the apical lesion, the interaction between bacteria and pro-inflammatory cytokines causing tissue destruction started from extracellular matrix degradation.<sup>8</sup> Many extracellular matrix components that support tissue integrity, cell migration regulation, as a source of cytokines and as a growth factor.<sup>8</sup> Extracellular matrix degradation is done by a group of a protein

called matrix metalloproteinase. MMP activity regulated by their inhibitor enzyme called tissue inhibitor of matrix metalloproteinase (TIMP) that bind MMP with high affinity. In physiologic condition, TIMP binds MMP with ratio 1:1.<sup>9,10</sup> Imbalance between MMP and TIMP related to many pathologic conditions including periodontitis, rheumatoid arthritis and cancer which in general related to the increased level of MMP.<sup>11,12</sup> Kind of MMP that significantly relate to the pathogenesis of dental tissue destruction is MMP-9. High level of MMP-9 associated with chronic inflammation process in periapical. In contrast, a high level of TIMP expressed in the periapical healing process in human. Expression and activities of some MMP also regulated by growth factors, including epidermal growth factor by gene induction.<sup>11,12</sup>

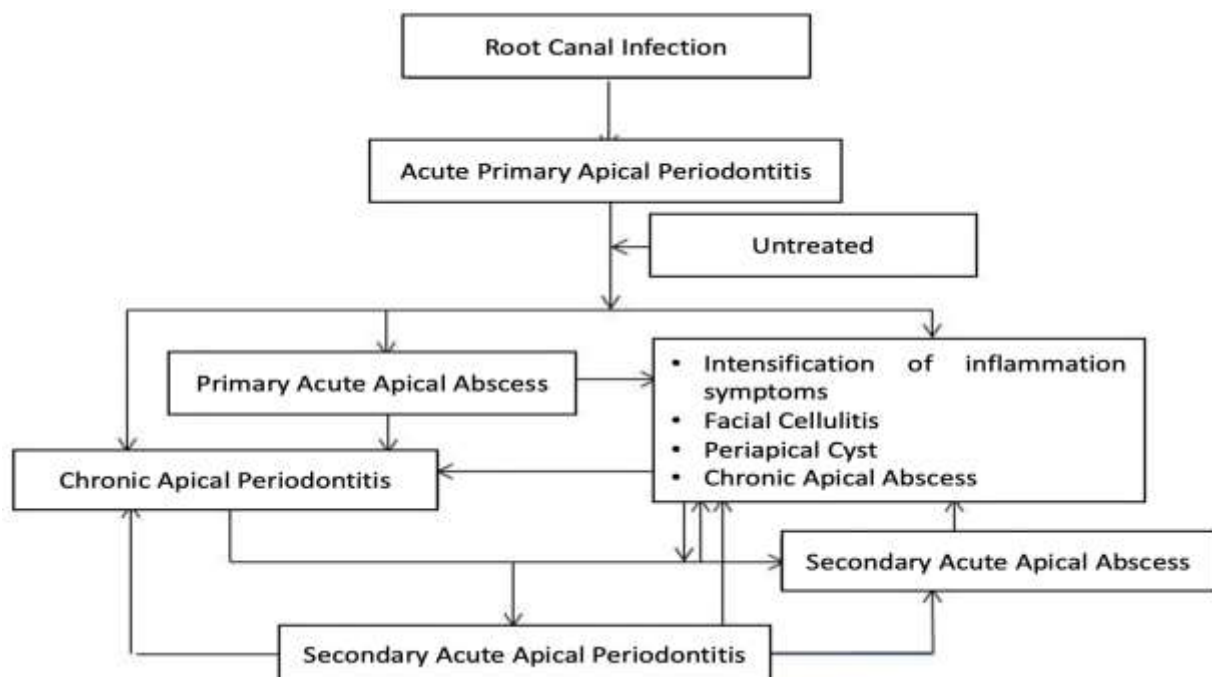
Some studies showed that there was a significant decreasing level of inflammatory mediators MMP-9, TIMP, and EGF after root canal filling procedure in root canal infection using intracanal medicament.<sup>13,14</sup> Based on the mentioned above, this review will discuss further the effect of intracanal medicament

using calcium hydroxide in decreasing level of MMP-9, TIMP-1 and EGF in the periapical lesion with root canal infection.

## Pathogenesis of Periapical Disease

Root canal infection is one kind of periodontitis, which is an inflammatory disease characterised by periodontal ligament destruction, root cerebrum and alveolar bone as tissue responses toward microbial plaque accumulation in dental root surface.<sup>1</sup> Root canal infection generally started with continuing dental caries. Root canal infection started by microbial invasion, colonisation, multiplication and pathogen activity.<sup>1,2</sup>

Initially untreated dental caries occurred and attain pulp tissue, causing extensive destruction in the pulp tissue and causing necrosis. The most common bacteria that found in root canal infection is anaerobic bacteria. In addition, the microaerophilic, facultatively anaerobic, and innovative anaerobic were also found.<sup>8</sup> A brief pathogenesis pathway of root canal infection to the periapical disease was shown in Figure 1 based on the several literatures.<sup>1,15,16</sup>



**Figure 1: Periapical disease pathogenesis that started with root canal infection<sup>1,15,16</sup>**

Root canal infection started by the production of biofilm layer in teeth and proximal of gingiva.<sup>9</sup> Biofilm layer contains lipopolysaccharide and then reach gingival tissue and induce immune and inflammatory responses.<sup>9</sup> These process was causing released of inflammatory mediators such as

cytokines, prostaglandins, free radicals and also matrix metalloproteinase (MMP). These pro-inflammatory mediators produced through a complex cascade signalling pathway that causing gingival tissue and alveolar bone destruction in the surrounding area.<sup>15,16</sup> Root canal infection also causing

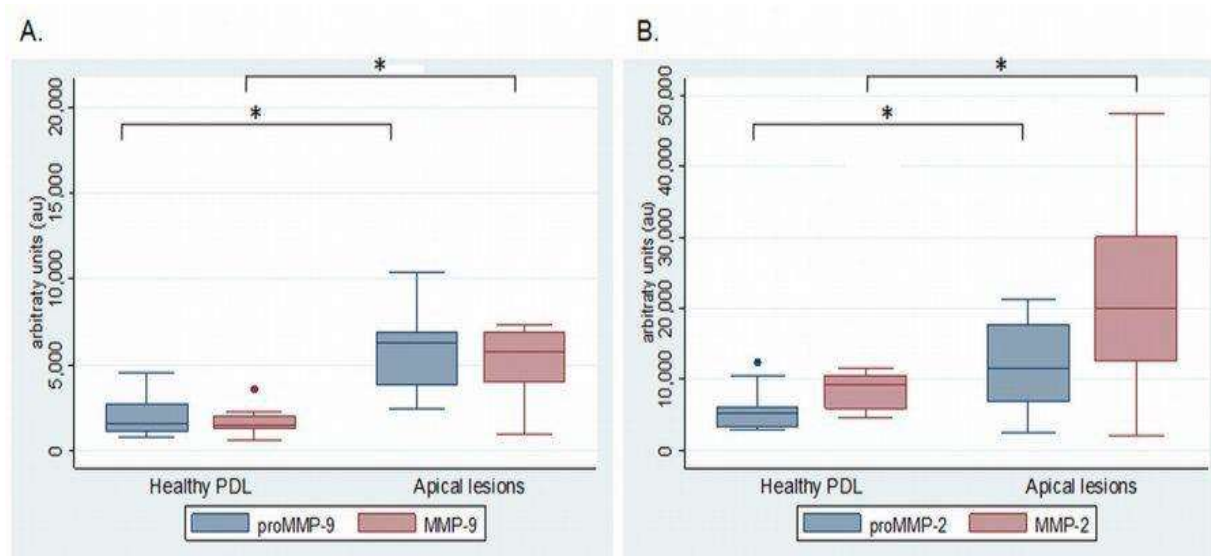
apical lesion that characterised by inflammation process and peri-radicular tissue destruction as a result of interaction between microbial factors and hosts inflammatory responses.

### Role of Pro-inflammatory Cells in Matrix Extracellular Destruction

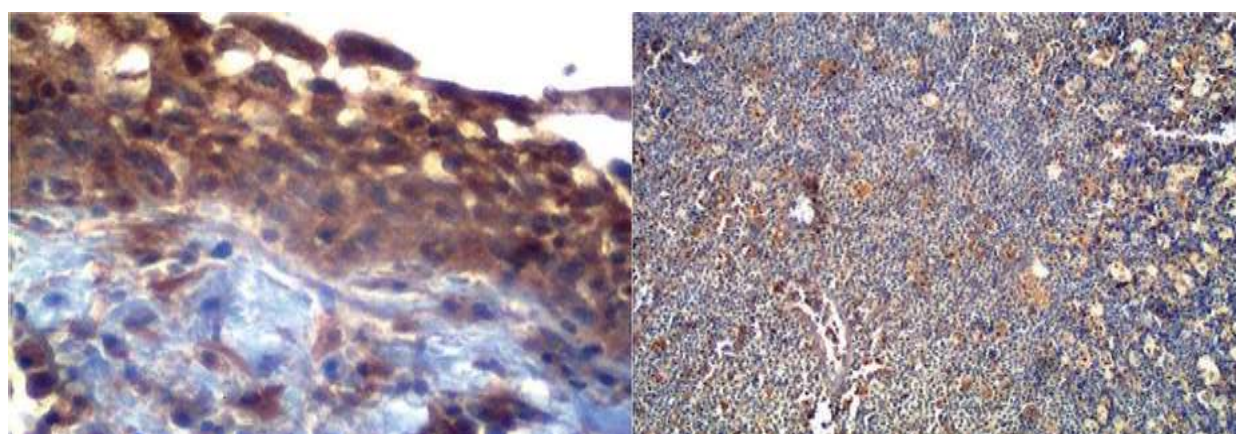
A study showed that oxidative imbalance and increasing level of MMP plays an essential role in development and progressivity of apical lesion formation.<sup>17</sup> Research also showed there is overexpression of MMP-9 in mRNA of granuloma type apical lesion.<sup>18</sup> This finding stated that MMP-9 contributes to the pathogenesis of apical lesion. Release of inflammatory mediators induces by macrophage to produce matrix metalloproteinase, which also contributes to extracellular matrix degradation.<sup>16</sup>

The activity of those inflammatory mediators is a response of microbial activity and also stimulate up-regulation of prostitution enzymes, one of them is matrix metalloproteinase. Inflammation process in apical tissue destruction also mediated by TNF- $\alpha$  and IL-1 $\beta$  which directly related to infection severity in root canal system.<sup>17-20</sup>

Number of studies found that decreasing level of MMP in root canal infection after therapy with intracanal medicament using calcium hydroxide paste.<sup>19-21</sup> These findings indicating that analysis of MMP-9 after endodontic treatment is needed to evaluate treatment outcomes after evaluation of pro-inflammatory cells activity as shown from the previous study (Figure 2 and 3)<sup>20,21</sup>.



**Figure 2: The comparison of activity level between MMP-9 (A) and MMP-2 (B) in apical lesion and healthy teeth. Activity MMP found to be higher in teeth with apical lesion.<sup>21</sup>**



**Figure 3: The expression of MMP-9 in apical lesion with immunohistochemistry staining (A) MMP-9 expression in radicular cyst (B) MMP-9 expression in periapical granuloma<sup>20</sup>**



## Intracanal Medicament in the Treatment of Root Canal Infection

Treatment of root canal infection needs intracanal medicament that simultaneously eliminates bacteria, slow its growth and stop spreading and cut of its nutritional supply.<sup>9</sup> There are many intracanal medicaments used in endodontic treatment, and the most common is calcium hydroxide Ca(OH). Calcium hydroxide has an excellent antibacterial effect through breakdowns of ion Ca<sup>2+</sup> and OH<sup>-</sup>.<sup>5</sup> Advantages of calcium hydroxide compared with another medicament material are bactericidal continue with bacteriostatic effect, accelerate healing and regeneration process of the root canal system, it's high pH-induced fibroblast production, impede internal resorption, neutralising acid pH caused by bacteria activity and also it's affordable price and easy to use.<sup>9,13</sup>

Before use, calcium hydroxide needs to be combined with a solution so it can release the induction hormone.<sup>9,13</sup> Antibacterial effect of calcium hydroxide directly influenced by the number of ions OH<sup>-</sup> that released. Calcium hydroxide mechanism in killing microorganisms done by released on hydroxide ion, so there's increasing pH level.<sup>5</sup> High pH will destruct the bacterial cytoplasmic membrane and lead to protein denaturation.<sup>5</sup> Protein denaturation will suspend the replication process of bacteria DNA and stop its growth process. Besides that, calcium hydroxide also can hydrolyse lipid causing lowered bacteria toxicity, pirogenity, macrophage activity and complement cell activities. The third mechanism is calcium hydroxide's high pH can neutralise acidic condition, thus suppress inflammation reaction.<sup>5</sup> The other mechanism describes antimicrobial effectivity of calcium hydroxide in absorbing carbon dioxide in root canal system that important for microbes in root canal system such as *Capnocytophaga* and *Eikenella*.<sup>1</sup> Besides that, calcium hydroxide also has the ability in degraded rest pulp tissue so it can maximise clearing process of root canal system.<sup>5</sup>

As mentioned earlier, in root canal infection, the apical lesion formed mediated by pro-inflammatory cytokines.<sup>19,20</sup> Calcium hydroxide can decrease inflammatory

mediators that linked with root canal infection pathogenesis such as MMP-9, TIMP-1, and EGF.<sup>21,22</sup> Matrix metalloproteinase is a group of enzymes that needs zinc in their activation site for catalysis activation.<sup>21</sup> Extracellular matrix degradation by MMP is the first inducer for the inflammatory process. Inflammatory cytokines such as IL-1B and TNF $\alpha$  can stimulate MMP to release both directly and indirectly in the periapical region or periodontal and causing persistent inflammation process.<sup>19,20</sup>

Matrix metalloproteinase divided into some groups based on its substrate and locations.<sup>23</sup> Their types are collagenase group consisted of MMP-1, MMP-8, MMP-13, MMP-18.<sup>23</sup> Gelatinase group consisted of MMP-2 and MMP-9, stromelysin group consisted of MMP-3, MMP-10, MMP-11, and membrane-type MMP.<sup>23</sup> Matrix metalloproteinase 2 and 9 are the most commonly involved in the pathogenesis of pulp tissue destruction, periodontal, and periapical.<sup>24,25</sup> MMP-9 mostly involved in gelatine and collagen type IV degradation, which are part of the basement membrane. A study showed that MMP-9 expression found higher in infected root canal compared in healthy teeth.<sup>19</sup> A study by Ahmed GM et al. found a correlation between the presence of gram-negative bacteria with higher expression of MMP-9 in symptomatic apical lesion.<sup>26</sup> MMP-9 also plays a role in tissue resorption and destruction periapical tissue and extracellular matrix degradation. Degradation of connective tissue regulated by MMP which also involved in the gingival tissue and alveolar bone destruction in surrounding teeth.<sup>26</sup>

## The Effect of Intracanal Medicament to the Inflammation on Apical Lesion

A study showed a higher presentation of MMP-9 in a dental apical lesion that not received endodontic treatment using calcium hydroxide as intracanal medicament.<sup>26</sup> Intracanal medicament using calcium hydroxide lowering MMP-9 level in the apical lesion.<sup>19,20</sup> In teeth with an apical lesion that received calcium hydroxide once, or in the apical lesion that not received intracanal medicament, showed that mononuclear cell dominantly takes a role in MMP expression.<sup>19,20</sup> While in an apical lesion that received intracanal medicament using

calcium hydroxide, MMP-9 expression found same in mononuclear and fibroblast cell and also in lower level compared to the apical lesion that not received intracanal medicament.<sup>19,20,26</sup>

In teeth that received intracanal medicament using calcium hydroxide observed that inflammatory cell level is lower accompany by fibroblast cells in the extracellular matrix.<sup>10,13</sup> Beside that, MMP-9 expression also found lower compare with an apical lesion that not received calcium hydroxide.<sup>24-26</sup> Both findings indicated reduction synthesise of MMP in rich calcium condition. On a side note, lowered MMP level also causing expression of tissue inhibitor of MMP-1 (TIMP-1) found to be higher in the same condition. This finding indicated that calcium hydroxide could give in vivo inhibition effect to MMP.<sup>10,17,18</sup>

A histopathologic study showed a faster healing process in an apical and periapical lesion that received intracanal medicament using calcium hydroxide compared with an apical lesion that received the treatment.<sup>27</sup> Besides that, the apical lesion that received calcium hydroxide also has lower bacterial contamination percentage, lower level of MMP expression and more organised extracellular matrix compared with an apical lesion that only received one visit treatment.<sup>28</sup> These findings could be related to disorganised collagen fibres that occurred in apical lesion without calcium hydroxide, indicating that calcium hydroxide take a role in tissue healing process.<sup>19,20</sup>

Synthesise process and function of MMP regulated by transcriptional activation and the post-transcriptional process by endogen inhibitor group called tissue inhibitor of metalloproteinase (TIMP).<sup>29</sup> A balance between MMP and TIMP determined extracellular matrix condition, where the excess level of MMP or lack of TIMP will causing degradation of extracellular matrix and pointed into tissue destruction condition.<sup>30</sup> TIMP first discovered in 1975 through the culture of human serum and found to inhibit collagen activity.<sup>29</sup> TIMP form a stoichiometry complex with MMP by covalent bind with ratio 1:1, can inhibit almost all kinds MMP with different affinity.

TIMP-1 is a natural inhibitor that inactivated MMP.<sup>29</sup>

Generally, altered cytokine expression will influence balance level between MMP and TIMP. In healthy periodontal tissue, TIMP level found to be higher than TIMP level in inflamed periodontal tissue.<sup>31</sup> MMP level that higher than TIMP will be increasing along with disease severity. In periodontal disease, MMP-1,2,3 and nine increase in human saliva gingiva, in contrast, TIMP-1 and TIMP-2 significantly decrease compared with a healthy control group.<sup>32,33</sup> The increasing level of TIMP occurred two months after scaling and root planing therapy and related with decreasing level of MMP that binds to TIMP-1.<sup>32,33</sup>

In root canal treatment using intracanal medicament using calcium hydroxide study found declining ratio between MMP-9 and TIMP-1 with negative bacteria finding compared with persistent or positive bacteria finding.<sup>19-22</sup> We can conclude that decreasing ratio between MMP-9/TIMP-1 with negative bacteria finding indicating a higher concentration of TIMP-1 compared with persistent or positive bacteria finding and it can facilitate the healing process.<sup>19,30-33</sup>

Following biomarker that known has a decreasing level in apical lesion after treatment with calcium hydroxide is epidermal growth factor (EGF).<sup>33,34</sup> EGF is a small polypeptide involved in epithelial growth and periodontal tissue differentiation. EGF and EGF-receptor is a transmembrane tyrosine kinase protein activated through binding with growth factor localised in the dental follicle, alveolar bone and ameloblast where all of these findings related to their important role in the process before and after a dental eruption. EGF and EGF-receptor level found to be high in the healthy epithelial cell where fibroblast cells also expressed many EGF receptor.<sup>33,34</sup> EGF can induce many biologic activities like cell proliferation stimulation, cell differentiation and migration, proteinase production and inhibit gastric acid production. EGF also acts as an important regulator for extracellular matrix degradation because it can stimulate collagenase secretion, gelatinase, and plasminogen activator from any cells. Using immunohistochemistry technique, EGF and

EGF-receptor reported increasing during the gingiva tissue inflammation process thus EGF plays a role as an important mediator in periodontal disease.<sup>33,34</sup>

In apical lesion that received intracanal medicament using calcium hydroxide, the study reported that it could prevent excessive inflammation by decreasing MMP-9, TIMP-1 and EGF level.<sup>33,34</sup> MMP and TIMP-1 synthesise regulated extensively by many growth factors and cytokines, and there is coordination with extracellular protein. EGF can increasing regulation of TIMP-1, but also can induce MMP-9 activation through EGF and increasing cell motility. In inflammation process, EGF also takes a vital role in extracellular matrix degradation because EGF can stimulate collagenase secretion, gelatinase and plasminogen activator from many cells.<sup>33-35</sup> In the healing process, EGF can increase regulation of TIMP-1 by fibroblast to secrete many extracellular matrix components, increasing fibronectin synthesise thus EGF also acts as proliferation regulator in healing process.<sup>33-35</sup> However, in several circumstances, the healing process of root canal infection mediated by TIMP could be influenced by diabetes mellitus, malignancy, and other chronic inflammation biomarker.<sup>36-38</sup>

## Conclusion

In root canal infection with the apical lesion, an immune-inflammatory process mediated by proinflammatory cytokines such as MMP-9, TIMP-1, and EGF. Root canal treatment using intracanal medicament was commonly used by calcium hydroxide. Calcium hydroxide as an intracanal medicament in the apical lesion can reduce excessive inflammation and helps the dental tissue healing process by decreasing MMP-9, TIMP-1 and EGF level that occurred in complex biological proses.

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