ORIGINAL ARTICLE

The IFNγ Expression In Periapical Dental Granuloma Immunopathobiogenesis

*Risya Cilmiaty[1,2]

[1] Fakultas Kedokteran Program Studi Ilmu Kedokteran(S3).Jl. Ir Sutami 36A,Universitas Sebelas Maret, 57126 Kentingan Surakarta, Indonesia
[2] Hospital of Univeritas Sebelas Maret, 57126 Kentingan Surakarta, Indonesia

ABSTRACT

Background: Relapse and pain are often found after endodontic treatment of pulp necrosis accompanied by periapical granuloma. IFN-γ is known to have a role in macrophage activation, which in turn produces proinflammatory cytokines. However, the exact role of IFN-γ is still uncertain. **Methods:** The samples in this study were teeth with and without granuloma extracted from patients with pulp necrosis. X-rays were used to detect periapical lesions, followed by an immunohistochemical and histopathological examination to observe IFN-γ expression. **Results:** Periapical granuloma had a predilection for women and the age group of 36-45 years old (66.67% and 67.%, respectively). Higher expression of IFN-γ was found in necrotic teeth with granuloma than in non-granuloma ($p < 0.001$). **Conclusion:** The primary component in the formation of periapical granuloma in pulp necrosis is the increased expression of IFN-γ.

Keywords: Periapical granuloma, pulp necrosis, IFN-γ expression, necrotic teeth, periapical lesions

Corresponding Author:
Risya Cilmiaty
Email: risyacilmiaty@staff.uns.ac.id
Tel: 0271-7890444

INTRODUCTION

Microorganisms enter the root canal through dental caries that have reached the pulp chamber, which in turn can cause pulp necrosis. These microorganisms can further cause chronic inflammation that resulted in periapical lesions. These lesions can take many forms, including granuloma, abscess, and cysts. Inflammatory cells, such as macrophages, mast cells, plasma cells, and lymphocytes absorb the granulation tissue, which is the component found in these lesions.(1,2)

Macrophages, fibroblasts, monocytes, and other cells are attracted by this process in the immune system, which will produce proinflammatory cytokines that cause tissue damage and degradation of the components of extracellular matrix (ECM), leading to the resorption of periapical tissue.(3,4)

Periapical tissue resorption is considered bone resorption. Proinflammatory cytokines such as interferon-γ, interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) are produced by macrophages, which modulated bone resorption process as a response to infections in the root canals and periapical when nuclear factor kappa B (NF-κB) is activated. (6)

Plasma cells, macrophages, lymphocytes, and mast cells are the inflammatory cells that granulate and form a dental granuloma, as seen in histological examination. The major components in a periapical granuloma surrounded by a capsule of dense connective tissues are macrophages.(7) Activation and phagocytic function of macrophages occur during bacterial infection in the root canals. They also have another function as antigen-presenting cells (APC). This function works by processing antigens, then presenting them to the T-helper lymphocytes via MHC II (MHC II).(8) This, in turn, will result in painful inflammation of an acute granuloma.(9)

The function of periapical granuloma is to eliminate infections by way of immune cell exudation, which is used as a mediator for the production of inflammatory cells, such as cytokines. The responses of Th-1, Th-2, and Th-17 trigger inflammatory reactions, indicating disease progress and bone resorption. Increased proinflammatory cytokines are evident in the process of pathogenesis (2,3).

There is a lack of studies investigating the expression of IFN-γ in the periapical granuloma. Therefore, this study aims to compare the expression of IFN-γ between periapical granuloma and non-periapical granuloma accompanying pulp necrosis. The results of this study can be used to analyze the role of IFN-γ in the

immunopathogenesis of acute periapical granuloma following endodontic treatments. A better understanding of the pathogenesis will lead to a better therapy of IFN-γ suppression. Therapeutics in endodontic treatment in the future may include a suppressing agent for IFN-γ expression (as immunomodulators).

METHODS

This cross-sectional analytical observational study was conducted at Dr. Moewardi Dental Clinic, Surakarta, Central Java. The diagnosis of periapical granuloma was established through a histopathological examination after dental extraction.

Patient Criteria

The study subjects, with informed consent, were selected based on the following criteria:

1. 17-57 years old
2. Have no systemic disease
3. Not consuming antibiotics or immunosuppressants
4. Albumin levels ranging between 3.5–5.2 mg/dl
5. Hemoglobin levels ranging between 2.3-15.0 g/dl

Periapical Lesion Determination

The selection and determination of periapical lesions were carried out using the following criteria:

1. Periapical lesions in maxillary and mandibular permanent teeth with deep caries.
2. Periapical lesions are visible in radiographs and can be differentiated between periapical or non-periapical granuloma.
3. Periapical granuloma appears radiolucent with a clear apical border.
4. Non-periapical granuloma without the evidence of granuloma tissues in the microscope, but showing chronic inflammation. The histopathological examination indicated the presence of lymphocytes, plasma cells, mast cells, fibroblasts, and macrophages.
5. All of the teeth have pulp necrosis with the indication of extraction under the consent of the patients.

Hematoxylin-Eosin (He) Staining (6)

Periapical lesions, previously separated from teeth, were immersed in 10% buffer saline (Merck). The determination of periapical and non-periapical granuloma follows these criteria for histopathological examination:

1. Periapical granuloma is indicated by chronic inflammation consisting of granulation tissue, with fibrous tissue surrounding its walls. Lymphocytes, giant cells, plasma cells, mast cells, fibroblasts, and macrophages are also evident.
2. Non-periapical granuloma is indicated by chronic inflammation without granulation tissue. Lymphocytes, plasma cells, mast cells, fibroblasts, and macrophages are also evident.

IFN-γ Expression (6)

IFN expression was investigated with immunohistochemistry. HRP-DAB (Ultravision plus) R&D coloring kit was used for coloring per the manufacturer's instruction. Hematoxylin-eosin (HE) is used for staining with monoclonal antibody IFN-γ as the primary antibody.

A blind assessment of the images was carried out by two investigators. Five random areas around the periapical lesion in each specimen were selected for histopathological examination under 400x magnification. The mean was calculated. A different pathologist confirmed unsuitable cases at the end of the evaluation.

Statistical Analysis

The SPSS 20.0 software (SPSS Inc., Chicago, IL) was used to conduct an independent t-test to compare IFN-γ expression with a statistical significance of $P < 0.05$. This study was approved by the Ethics Committee of Sebelas Maret University (No: EC106/XI/2008) in accordance with the principles of the Declaration of Helsinki.

RESULTS

Gender predilection in periapical and non-periapical granuloma is presented in Diagram 1, with periapical granuloma occurring mostly in women (67%), and non-periapical granuloma in men (61.11%).

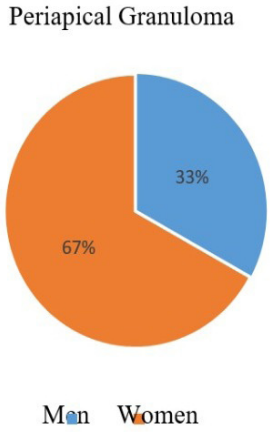


Diagram 1. Periapical Granuloma Patient Gender

Age predilection in periapical and non-periapical granuloma is presented in Diagram 2, with periapical granuloma occurring mostly within the age group of 18-65 years (100%), and non-periapical granuloma within the age group of 18-65 years (94%).

Patients' Lesion Specimens

The location of periapical and non-periapical granulomas is depicted in Diagram 3, where most periapical granulomas were located in the posterior region (94%), while non-periapical granulomas were also located in the posterior region (100%).

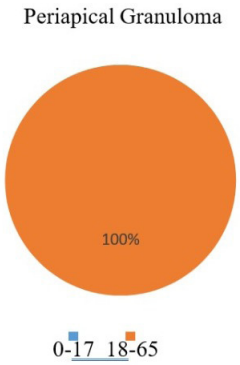


Diagram 2. Periapical Granuloma Patient Age

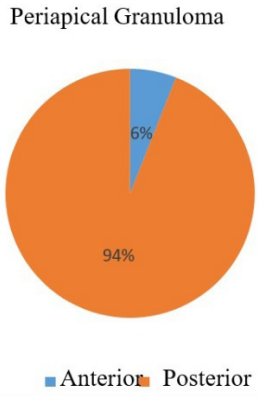
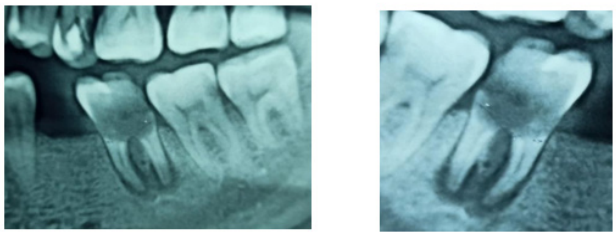


Diagram 3. Periapical Granuloma Location

Based on the periapical radiograph examination, all enamels were radiolucent to the dentine in the pulp chamber. This is an indication of a severe deterioration of the hard tissue of the tooth. The periapical granuloma was radiolucent with clear apical margins (Figure 1A). Non-periapical granuloma showed an enlargement by apical periodontitis with apical radiolucency (Figure 1B).



(A) (B)

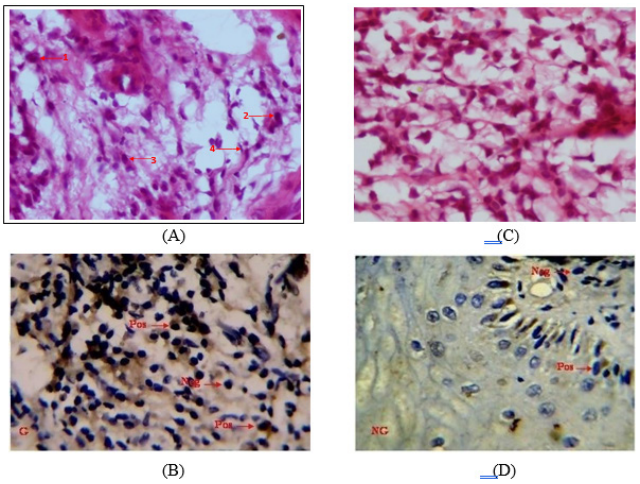
Figure 1. (A) Periapical Granuloma (B) Non Granuloma

Hematoxylin-Eosin Staining

Figures 2A and 2B presented the results of Hematoxylin-eosin (H&E) staining in the periapical and non-periapical granuloma. Granulation tissue was found in periapical granuloma, but not in the non-periapical granuloma. Both groups showed plasma cells, lymphocytes, macrophages, mast cells, and fibroblasts. Giant cells and datia cells were also found in periapical granuloma (Figs. 2A and B).

IFN-γ Expression

Based on immunohistochemistry, IFN-γ expression was found higher in periapical granuloma compared to non-periapical granuloma ($p < 0.001$). IFN-γ was also found in all periapical granulomas (Figs. 2C and 2D).



(A) (C)

(B) (D)

Figure 2. (A) Granuloma tissue (B) Non Granuloma (C) Periapical Granuloma (D) non Granuloma

Ethical Clearance

This study received approval from the Research Ethics Committee, Faculty of Medicine, Sebelas Maret University No. EC106/XI/2008.

DISCUSSION

Periapical granuloma was more common in women (66.67%) than men (33.33%), and also more common in subjects aged 36-45 years old (61.11%). This contradicts Omoregie et al. who showed that periapical granuloma was predominantly found in men aged 21-30 years old. (10)

Irreversible pulpitis, fibroblasts, and mast cell necrosis in the dental pulp were attributed to bacterial infection. This was in line with Omoregie et al., who stated that macrophages, some lymphocytes, and cytoplasmic foamy with or without neutrophils occupied the dominant cells during the early stages of periapical granuloma.(10)

The results showed that irreversible pulpitis or pulp necrosis with periapical granuloma had the highest distribution of bacteria and bacterial biofilms in the root canal system. The infection of bacteria triggered the immune system to release multiple mediators in the root canal, which initiated a reaction in the apical tissues, resulting in the formation of periapical lesions.(11)

Granulomatous tissue containing fibroblasts, a well-developed fibrous capsule, and inflammatory cells constituted periapical granuloma caused by pulp necrosis. In histopathological examination, 79.2% of lymphocytes, plasma cells, macrophages, and giant cells were detected in the periapical granuloma, 81.2% in cementum, and 65.6% in the apical surface of teeth with periapical granuloma. Infection of the root canal

usually attributes to endodontic treatment failure, which leads to the accumulation of bacteria and antigens that in turn lead to persistent apical periodontitis.(12)

Macrophages play a critical role in periapical granuloma development. The presence of bacteria promotes the development of histiocytes into macrophages. It leads to the production of HSP60, resulting in the apoptosis of CD4 lymphocytes (TH2 cells), shifting the balance to CD8 lymphocytes (TH1 cells). This is expressed by the increase of IFN-γ-producing cells from the induction of IL-12 that triggers the formation of granuloma. (13) Macrophages, a major component in periapical granuloma, activate antigen-presenting cells (APC) toward T lymphocytes to release proinflammatory cytokines, including IL-6, IL-11, IL-17, IL-1β, and TNF-α.(14,15) There was an evidence of higher IFN-γ expression in periapical granuloma compared to non-periapical granuloma in this study.

Based on the results of the study, cells are more capable to eliminate bacteria along with an increase in the immune system. Cytokines were also increased as a result of intracellular signals to deliver extracellular signals of IL-12 products. Furthermore, these cytokines will induce T lymphocyte to secrete IFN-γ, which activates macrophages and prevent the spread of bacteria by surrounding the cells containing intracellular bacteria with macrophages. This is known as the formation of granuloma.(16,17) Breloer et al. supported this theory and showed a significant increase in IFN along with the addition of Hsp60 to macrophages and T-cells. However, there was no increase in IL-2 production or T-cell proliferation. Macrophages' ability to produce IL-2 fully determines IFN-γ induction.(18)

Other cells found in periapical granulomas are mast cells, which are associated with bone tissue breakdown and periapical granuloma growth. Local osteolytic activity is increased by the production of cytokines from the activation of IL-1, IL-6, mast cells, and especially TNF-α.(19)

Sasaki et al. revealed the involvement of IL-2, IL8-, and IFN-γ in infections that induce bone resorption, which described an increase of macrophages during the beginning of periapical infiltration.(20) These macrophages produced the cytokines involved in bone resorption, mainly IL-1 and TNF-α. They also produced IL-12 and IL-18 that act in the differentiation of naпve T-cells into Th-1, resulting in the production of IFN-γ and the induction of NK cells that produce IFN-γ. IFN-γ is involved in the suppression and increase of macrophage activities, induction of IL-1, NO synthase, and O2-. NFK-β ligand-receptor activator (RANKL) will be suppressed by IL-12, along with IFN-γ and IL-18. (19,20)

Granuloma is a chronic inflammatory process due to the failure of an acute inflammatory process. Persistent antigens cause continuous activation and accumulation of macrophages. Macrophages are transformed into epithelioid cells and datia cells (a fusion of several macrophages) by IFN-γ, which was released by T cells. (17) Increased macrophage activity to surrounding macrophages that contain intracellular bacteria was evident in necrotic teeth accompanied by periapical granuloma, although there was no destruction of macrophage cells containing intracellular bacteria.(16)

This study highlighted high IFN-γ expression in periapical granuloma, mainly constituting lymphocytes, mast cells, and macrophages with a high recurrence rate following endodontic therapy. Hence, adequate endodontic treatment is desirable in cases of irreversible pulpitis or pulp necrosis accompanied by periapical granuloma to prevent the production of proinflammatory cytokines and the activation of IFN-γ.

CONCLUSION

Increased IFN-γ expression is present in necrotic teeth involving periapical granuloma. Further comprehension of the progression of periapical lesions can be reinforced by understanding the involvement of IFN-γ in the pathogenesis of periapical granulomas.

ACKNOWLEDGEMENTS

The author would like to thank the Faculty of Medicine UNS and those who have assisted in the research.

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