

Inflammatory Cytokine Serum Levels in Sockets Following Extraction of Teeth with Apical Periodontitis

Meiny F. Amin¹, Ratna Meidyawati², Melanie S. Djamil³, Benny S. Latief^{4*}

1. Doctoral Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

2. Department of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

3. Department of Biochemistry and Molecular Biology, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia.

4. Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

Abstract

Porphyromonas endodontalis triggers macrophages and T-lymphocytes to release inflammatory cytokines serum level, including interleukin-1 β (IL-1 β), IL-6, IL-17), and tumor necrosis factor- α (TNF- α). *P. endodontalis* is one of the bacteria that causes apical periodontitis (AP).

To investigate of various inflammatory cytokines serum levels (IL-1 β , IL-6, IL-17, TNF- α) in socket blood following tooth extraction due to AP. We performed an observational analytical study on 10 teeth with AP compared with 10 normal teeth without periodontitis.

The teeth were extracted and blood was extracted from the socket using foam, squeezed in a 2 mL Eppendorf tube, and centrifuged to separate the serum. Enzyme-linked immunosorbent assay (ELISA) was used to measure all the inflammatory cytokine serum levels.

On t-test, IL-1 β levels in the socket blood following extraction of teeth with AP (4344.00 ± 2196.00 pg/mL) were significantly higher ($t = 6.71$; $p < 0.05$) compared to the controls (520.80 ± 213.70 pg/mL); IL-6 levels in AP (88.99 ± 35.66 pg/mL) were significantly higher ($t = 8.99$; $p < 0.05$) compared to the controls (13.70 ± 7.07 pg/mL); IL-17 levels in AP (68.50 ± 38.90 pg/mL) were significantly higher ($t = 5.07$; $p < 0.05$) compared to the controls (8.19 ± 3.82 pg/mL); and TNF- α levels in AP (1816.00 ± 966.30 pg/mL) were significantly higher compared to the controls (307.10 ± 154.60 pg/mL) ($t = 6.19$; $p < 0.05$).

The level of inflammatory cytokines serum in the socket following tooth extraction increases in teeth with AP.

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Introduction

For centuries, a relationship has been suspected between oral infection and systemic disease. Oral health effects on the human body were proposed by the Assyrians in 7th century BC. In the 18th century, Benjamin Rush, a doctor from the U.S. suggested that arthritis can be healed in some people following removal of infected teeth. After several decades, various scientific cases have shown a link between oral infections (viruses, bacteria, yeast) and systemic diseases (atherosclerosis, cardiovascular disease, cerebrovascular

disease, premature and low birth weight, pulmonary disease) and between systemic diseases (arthritis, diabetes, HIV infection, and osteoporosis) and oral, dental, and orofacial diseases.¹

Epidemiological studies show that one of the factors that has biologic potential as a cause of atherosclerosis is a low degree of infection in the oral cavity.² The present study reported a relationship between poor oral health and cardiovascular disease,³ other risk factors are lifestyle, smoking habits, obesity, physical activity, psychosocial influence, and diet. Many authors have responded to this opinion, and most studies support the alleged link between poor oral health and atherosclerosis although some epidemiological studies show no association.

Many risk factors for systemic diseases have been identified. Recent evidence shows that chronic inflammation is the etiology of systemic diseases, especially cardiovascular

*Corresponding author:

Benny S. Latief

Department of Oral Biology and Maxillofacial Surgery,
Faculty of Dentistry, Universitas Indonesia,
Jakarta, Indonesia.

E-mail: bennylatief@gmail.com

disease.⁴ Low-grade chronic infections, such as periodontal disease and apical periodontitis (AP), are often found in the oral cavity. Periodontal disease can be considered a risk factor for cardiovascular disease; while AP is also regarded as a risk factor for atherosclerotic cardiovascular disease.² Chronic low-grade oral cavity infections are associated with an increase in several chronic inflammatory markers. Chronic inflammation is also associated with increased systemic inflammatory markers, including tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8).⁵

AP in the root canal and periapical tissue is also a chronic low-degree infection and is also considered a polymicrobial infection. Bacteria commonly found in teeth with pulp necrosis are *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Porphyromonas endodontalis*.⁶ *P. endodontalis* is a dark-pigmented, gram-negative bacterium that is present in the infected pulp and is pathogenic to the pulp. *P. endodontalis* was found in 50% of microbial root canal samples of teeth with necrosis and primary endodontic infection.⁶

The role of *P. endodontalis* in the pathogenesis of endodontic disease is quite extensive because the outer membrane contains lipopolysaccharide (LPS), which plays a major role in the inflammatory process by inducing an immunoinflammatory reaction, resulting in an increase in levels of inflammatory cytokines such as IL-1 β , IL-6, IL-17, TNF- α .⁷⁻⁹ *P. endodontalis* LPS has a higher potential compared to the LPS of other gram-negative bacteria found in periodontal tissues and a very high percentage is found in endodontic infections.^{6,7} *P. endodontalis* LPS triggers macrophages and T-lymphocytes to secrete inflammatory cytokines serum. In this study, we examined the levels of various inflammatory cytokines serum (IL-1 β , IL-6, IL-17, TNF- α) in the socket blood following tooth extraction for AP. This study determined of inflammatory cytokine serum level can be detected in socket following extraction of teeth with apical periodontitis.

Research methods

We performed an observational analytical study in 10 posterior teeth with AP and 10 controls with normal teeth without AP indicated for extraction orthodontic treatments or impacted teeth. The ethical clearance of this study declared by ethical medic community Trisakti University.

Following the method of Roeslan et al., the teeth were extracted, blood was taken from the socket using foam¹⁰ and then squeezed into a 2 mL Eppendorf tube. The procedure was repeated until the required sample size (2 mL) was obtained and, then, was centrifuged to separate the serum and put in -20⁰ C freezer.

Enzyme-linked Immunoabsorbent assay (ELISA Abnova) was used to measure all the levels of inflammatory cytokines (IL-1 β , IL-6, IL-17, TNF- α) in the serum. The component of the Elisa Kit for determination of cytokines were presented in table 1. A total of 100 μ L of IL-6 standard and 50 μ L of sample were distributed to plate. Next, the diluted 50 μ L of IL-6 enzyme conjugate reagent was distributed to each plate and incubated at 18^o-25^oC for 2 hours. The microtiter plate was emptied and rinsed with wash buffer, which was diluted by adding 400 μ L to each well and, then, the plate was emptied again. The area was flushed 4 times. The plate was dried using a tissue, then, 100 μ L of streptavidin-HRP which had been diluted on each plate was added and, then, was incubated at room temperature for 1 hour.

The microtiter plate was emptied and rinsed with a wash buffer that was diluted by adding 400 μ L to each well and, then, the plate was emptied again. Flushing was done 4 times. A total of 100 μ L of TMB substrate solution was added to each plate, incubated at room temperature for 10 minutes, and 100 μ L of stop solution, which was given to stop the enzymatic reaction, was added. Color changes were read using a spectrophotometer compared to the standard curve of each inflammatory cytokine.

Data were calculated by using a statistical program and presented as means and standard deviation. The normality distribution of all data group was analyzed by using Saphiro-wilk test. All data passed the normality test were tested for significant differences by using Student t-test. Significant was accepted at p<0.05.

Cytokine	Antibody	Lot Number
Interleukin 1- β	Anti-human IL-1 β	1141253706
Interleukin-6	Anti-human IL-6	RN-55305
Tumor necrosis factor- α	Anti-human TNF- α	131556030
Interleukin-17	Anti-human IL-17	RN- 55310

Table 1. Component of the Elisa Kit for determination of cytokine

Results

Table 2 shows statistical testing of all the variables between inflammatory cytokines in AP cases and controls using *t*-test. Significant differences ($t = 6.71$; $p < 0.05$) were seen on *t*-test between levels of IL-1 β in the blood socket after tooth extraction in patients with AP and controls. The level of IL-1 β in AP (4344.00 ± 2196.00 pg/mL) was higher than in the controls (520.80 ± 213.70 pg/mL). Moreover, there were

significant differences ($t = 8.99$; $p < 0.05$) between IL-6 AP levels and the controls. IL-6 levels in AP (88.99 ± 35.66 pg/mL) were higher than in the controls (13.70 ± 7.07 pg/mL). Likewise, the levels of IL-17 in AP (68.50 ± 38.90 pg/mL) were significantly different ($t = 5.07$; $p < 0.05$) from the controls (8.19 ± 3.82 pg/mL). Table 2 also shows that the TNF- α levels in AP (1816.00 ± 966.30 pg/mL) were significantly higher than in the control (307.10 ± 154.60 pg/mL) ($t = 6.19$; $p < 0.05$).

Variable	Group	Mean \pm Standard Deviation (pg/mL)	t	p
IL-1 β	Apical periodontitis	4344.00 \pm 2196.00	6.71	0.000
	Control	520.80 \pm 213.70		
IL-6	Apical periodontitis	88.99 \pm 35.66	8.99	0.000
	Control	13.70 \pm 7.07		
TNF- α	Apical periodontitis	1816.00 \pm 966.30	5.07	0.000
	Control	307.10 \pm 154.60		
IL-17	Apical periodontitis	68.50 \pm 38.90	6.19	0.000
	Control	8.19 \pm 3.82		

Table 2. Results of *t*-test between levels of inflammatory cytokines serum in the socket following extraction of teeth with apical periodontitis and controls

Discussion

AP is a pathological state of dental periapical tissue characterized by alveolar bone destruction in the apical area of the tooth. AP starts from caries that are not properly treated; therefore, the pulp is irritated and inflamed due to bacterial infection. If the protective effect of the immune response is not enough, pulpitis will develop into chronic periapical lesions so that it is only able to localize and prevent further damage.¹¹ Further invasion of microorganisms or their products into the periapical tissues of the root canal will result in AP.

Untreated pulp inflammation can cause the pulp to become necrotic, after which bacteria in the pulp enter the periapical tissue through the apical foramen.¹² In infectious conditions, the body's bacteria will provide an immune response to fight the infection. When AP occurs, LPS gram-negative bacteria, such as *P. endodontalis*, trigger the secretion of various inflammatory cytokines by macrophages, Th2 lymphocytes, and stromal cells. Allegedly, inflammatory cytokines serum (IL-1 β , IL-6, IL-17, TNF- α) in the blood socket will increase after tooth extraction.

The present study showed that blood in the socket after tooth extraction will quickly clot; therefore, using a syringe to draw blood is difficult. To overcome this problem, taking blood

samples from the tooth extraction socket is done using a $2 \times 1 \times 1$ cm³ sterile sponge that has been soaked in EDTA and, then, dried.¹⁰ A sponge was chosen to absorb the blood because it has good absorption, with one piece of sponge absorbing up to 0.5 mL of blood. Using foam as a blood storage medium follows the research conducted in 1977 by Roeslan et al.¹¹ In that study, foam containing EDTA was used as the sampling media.

The technique of measuring the levels of inflammatory cytokines serum is performed by sandwich ELISA. The advantage of sandwich ELISA is antibodies are used specifically to bind to certain antigens; therefore, this method is easier to perform, and it is more sensitive and accurate compared to other ELISA tests.¹³

Inflammatory cytokine levels in the socket blood after tooth extraction in the present study demonstrated higher levels in AP than in the controls. This can happen because *P. endodontalis* in the periapical blood in the case of AP will release LPS, which stimulates macrophages, and Th lymphocytes secrete some chronic inflammatory markers such as TNF- α , IL-1 β , IL-6, and IL-17.^{4,9} These results are consistent with those by Elsalhy et al. (2013)¹⁴, who measured levels of various inflammatory cytokines in pulp blood in cases of irreversible pulpitis. If irritants cannot be removed,

neutrophils will gradually be replaced by macrophages, lymphocytes, and plasma cells in the collagen connective tissue. Proinflammatory cytokines derived from macrophages (IL-1, IL-6, and TNF- α) are potential stimulators for lymphocytes.¹⁶

The high levels of inflammatory cytokines are directly in proportion with the level of pulp inflammation.¹⁵ Barkhodar (1999)¹² found that levels of inflammatory cytokines, especially IL-6 in periapical tissue and inflamed pulp tissue in AP, were higher when compared to IL-6 levels in pulp tissue diagnosed with pulpitis and tissue taken during third molar extraction. The results of his research also indicate that IL-6 is produced and released locally in pulp and periapical tissues as are inflammatory cytokines. The confounding factors in this study law degree chronic infection such as gastritis, other periodontitis were excluded.

Conclusions

In the case of teeth with AP, the serum level of inflammatory cytokines in the socket blood increases after tooth extraction. The increase of cytokine serum level found locally in the tooth socket following extraction of teeth with apical periodontitis might imply to the increase of cytokine level systematically.

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Declaration of Interest

The authors report no conflict of interest.

References

1. Cohen DW, Slavkin HC. Periodontal Disease and Systemic Diseases, in *Periodontal Medicine*. Rose LF, Genco RJ, Cohen DW, Mealy BI (eds.). B.C. Decker, St. Louis 2000: 1-10.
2. Cotti E, Dessi C, Piras A, Mercurio G. Can A Chronic Dental Infection Be Considered A Cause of Cardiovascular Disease? A Review of The Literature. *Int J Cardiol*. 2011;148(1):4-10.
3. Carramolino-Cuéllar E, Tomás I, Jiménez-Soriano Y. Relationship Between The Oral Cavity and Cardiovascular Diseases and Metabolic Syndrome. *Med Oral Patol Oral Cir Bucal*. 2014;19(3):289-94.
4. Aarabi Ghazal, Heydecke G, Seedorf U. Roles of Oral Infections in Pathomechanism of Atherosclerosis. *Int J Mol Sci*. 2018;19(7):1978.
5. Elsalhy M, Azizieh F, Raghupathy R. Cytokines as Diagnostic Markers of Pulpa Inflammation. *Int Endod J*. 2013;46(6):573-580.
6. Cao H, Qi Z, Jiang H, Zhao J, Liu Z, Tang Z. Detection of *Porphyromonas endodontalis*, *Porphyromonas gingivalis* and *Prevotella intermedia* in Primary Endodontic Infections in A Chinese Population. *Int Endod J*. 2012;45(8):773-81.
7. Mirucki CS, Abedi M, Jiang J, et al. Biologic Activity of *Porphyromonas Endodontalis* Complex Lipids. *J Endod*. 2014;40(9):1342-8.
8. Azuma MM, Samuel RO, Gomes-Filho JE, Dezan-Junior E, Cintra LT. The Role of IL-6 on Apical Periodontitis: A Systematic Review. *Int Endod J*. 2014;47(7):615-21.
9. van der Wall SV, Lappin DF, Crielaard W. Does Apical Periodontitis Have Systemic Consequences? The Need for Well-Planned and Carefully Conducted Clinical Studies. *Br Dent J*. 2015;218(9):513-6.
10. Roeslan BO, Lestari D, Sadono MH. Serum IgG Levels in the Sockets of Teeth with Periapical Granulomas. *J Dent Res*. 1997;76:1209-21.
11. Graunaite I, Lodiene G, Maciulskiene V. Pathogenesis of Apical Periodontitis: A Literature Review. *J Oral Maxillofac. Res* 2012;2(4):e1.
12. Barkhodar RA, Hayashi C, Hussain MZ. Detection of Interleukin-6 in Human Dental Pulp and Periapical Lesions. *Endod Dent Traumatol*. 1999;15(1):26-7.
13. Gan SD, Patel KR. Enzyme Immunoassay and Enzyme-Linked Immunosorbent Assay. *J Invest Dermatol*. 2013;133(9):e12.
14. Elsalhy M, Azizieh F, Raghupathy R. Cytokines as Diagnostic Markers of Pulpal Inflammation. *Int Endod J*. 2013;46(6):573-80.
15. Erta M, Quintana A, Hidalgo J. Interleukin-6, A Major Cytokine in the Central Nervous System. *Int J Biol Sci*. 2012;8(9):1254-66.