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Original article

The Role of Interleukin-8 in the Development and Clinical Progression of Chronic Periapical Lesions

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SUMMARY

Introduction/Aim. Chronic apical periodontitis represents dynamic continuation of the presence of endodontic infection in the root canal system of the tooth, when the innate and acquired immune responses are activated and various cells and inflammatory mediators are recruited, which cause the consequent destruction of periapical tissues and the development of periapical lesions. The aim of the study was to analyze the concentration of IL-8 in tissue homogenates of periapical lesions and to compare the obtained results with the symptomatology of the patients and the size of the lesion.
Methods. A total of 93 tissue samples of chronic periapical lesions were analyzed in this study. In relation to the clinical symptoms, the samples were divided into symptomatic and asymptomatic, and according to the size, into large and small lesions. The concentration of IL-8 was examined using an ELISA test.
Results. The results showed a significantly higher concentration of IL-8 in symptomatic periapical lesions compared to asymptomatic ones ($p < 0.001$). The concentrations of this chemokine was also significantly higher in the large lesions when compared to the small ones ($p < 0.001$).
Conclusion. The elevated concentration of IL-8 in periapical lesions with pronounced clinical symptomatology as well as in large lesions specify that IL-8 is a dominant chemokine that contributes to the development of periapical inflammation and clinical progression of periapical lesions.

Keywords: **periapical lesions, cytokines, chemokines, IL-8**

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INTRODUCTION

Chronic apical periodontitis represents dynamic continuation of the presence of endodontic infection in the root canal system (1). It is caused by continual localized inflammation in the periapical tissues that leads to bone resorption and development of a periapical lesion, and represents one of the first oral pathologies where the involvement of the immune system has been proven in the formation and preservation of the inflammatory lesion. Apical periodontitis is the result of a complex interplay between microbes that cause endodontic infection in the canal system and the immune response of the host, when the innate and acquired immune responses are activated and various cells and inflammatory mediators are recruited. That complex mechanism will cause the consequent disintegration of periapical tissues and development of periapical lesions (2).

Macrophages are the first cells that come across the antigen upon entering the body, which phagocytose the particle. They, then release chemokines, including interleukin 8 (IL-8), in order to signal other immune cells to reach the area of inflammation. In this way, IL-8 has the role of a chemical signal that attracts neutrophils to the inflammation site and according to that is known as a neutrophil chemotaxic factor (3). This chemokine is one of the main mediators of the periapical inflammatory response. It is produced by a number of cell types, such as monocytes/macrophages and fibroblasts and is controlled by IL-1β and TNF-α. The main feature of the acute phase of apical periodontitis is the great infiltration of neutrophils, where IL-8 and other chemokines are especially important (4). Despite the fact that neutrophil granulocytes represent the primary target cells for IL-8, the wide range of cells (endothelial cells, macrophages, mast cells, keratinocytes) also react to this chemokine. The fact that the secretion of IL-8 is increased during oxidative stress and, on the contrary, by recruiting inflammatory cells, IL-8 causes a further increase in mediators of oxidative stress, making this chemokine a key parameter of localized inflammation (5).

The purpose of the study was to analyze the concentration of IL-8 in tissue homogenates of periapical lesions and to compare the obtained results with the symptomatology of the patients and the size of the lesion.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of the Faculty of Medicine in Niš, Serbia (no. 01-2066-5). It included 93 patients of the Clinic of Dental Medicine in Niš, Serbia, who were, according to appropriate clinical and radiographic methods, diagnosed with chronic apical periodontitis. For the analysis in the study, periapical lesions were taken exclusively from the roots of teeth which, due to the impossibility of treatment, were indicated for extraction.

Periapical lesions of the teeth that did not present any form of marginal periodontitis were included in the research. The condition for the inclusion of patients in the study was that they had not taken antibiotic and anti-inflammatory therapy in the previous two months, as well as that they did not suffer from acute or chronic illnesses that bring to a state of immunodeficiency. Anamnestic data were taken from each patient about the course and duration of the disease, symptoms and medication, and the eventual existence of local swelling or lymph gland swelling was determined by clinical examination.

According to the patients' subjective symptoms, the examined lesions were divided into two groups: symptomatic lesions (they were identify by swelling, pain, discomfort when chewing or sensitivity to percussion and palpation) and asymptomatic (they did not show acute symptoms at the time of the study) lesion. Periapical lesions were measured using ruler and, according to size, divided into two groups: small (≤ 5 mm) and large (> 5 mm) (Table 1). Given that periapical lesions include reactive tissue consisting mainly of inflammatory granulomatous tissue replacing periapical bone, there was no real tissue equivalent to serve as a negative control.

Table 1. *Division of the examined lesions into groups according to clinical symptoms and size*

	Large lesions	Small lesions	Total
Symptomatic lesions	23	23	46
Asymptomatic lesions	23	24	47
Total	46	47	93

Before the administration of the local anesthetic, each patient rinsed mouth with 0.12% chlorhexidine for 30 seconds, and additionally, the teeth indicated for extraction, surrounding gingiva and mucous membrane were cleaned with 0.12% chlorhexidine. Immediately after tooth extraction, the sterile scalpel was used to cut periapical lesions from the tooth root apex. Samples were rinsed in sterile physiological solution, dried on sterile cotton, put in a sterile plastic Eppendorf tube and then frozen at -70 ^{0}C. Tissue homogenization was performed using a Teflon crusher in ice cold phosphate buffer pH 7.4, and the amount was balance to the weight of the tissue, so that the obtained concentration of the tissue homogenate was 10%. Coarse detritus was sedimented by centrifugation at 1400 rpm for 1 minute at -40 ^{0}C. The supernatant was then frozen at -70 ^{0}C until appropriate biochemical analyses.

The analysis of the IL-8 concentration was performed using an ELISA test (R&D Systems Inc. Minneapolis, USA). The sensitivity of the ELISA test for IL-8 was 1.5 - 7.5 pg/ml.

Statistical analysis was performed using the Mann-Whitney Rank Sum test, using Sigmastat and Origin computer software. Results are shown as mean ± standard deviation. A value of 0.05 was determined as statistically significant.

RESULTS

Evaluation of the IL-8 concentration in tissue homogenate of chronic periapical lesions showed a significant concentration of this chemokine in all samples. Figure 1 presents the values of IL-8 in all lesions, and the average concentrations were analyzed in relation to clinical symptoms and the size of the lesions. The average concentration in the group with symptomatic lesions was 2231.54 pg/ml, whereas the average value in the group with no symptoms was 1119.37 pg/ml. There was a statistically significant variation in IL-8 levels between lesions with symptoms and those without ($p < 0.001$). The average IL-8 concentration in the group with large lesions was 2166.14 pg/ml, whereas the average value in the group with small lesions was 1189.56 pg/ml. Statistical analysis revealed that the group with large lesions had a significantly greater concentration of IL-8 ($p < 0.001$).

The mean IL-8 concentration values for each group of symptomatic and asymptomatic lesions are displayed in Figure 2. Within the symptomatic lesions group, the difference in IL-8 levels between large and small lesions was examined. In symptomatic large lesions, the average concentration of IL-8 was 2905.13 pg/ml, whereas in symptomatic

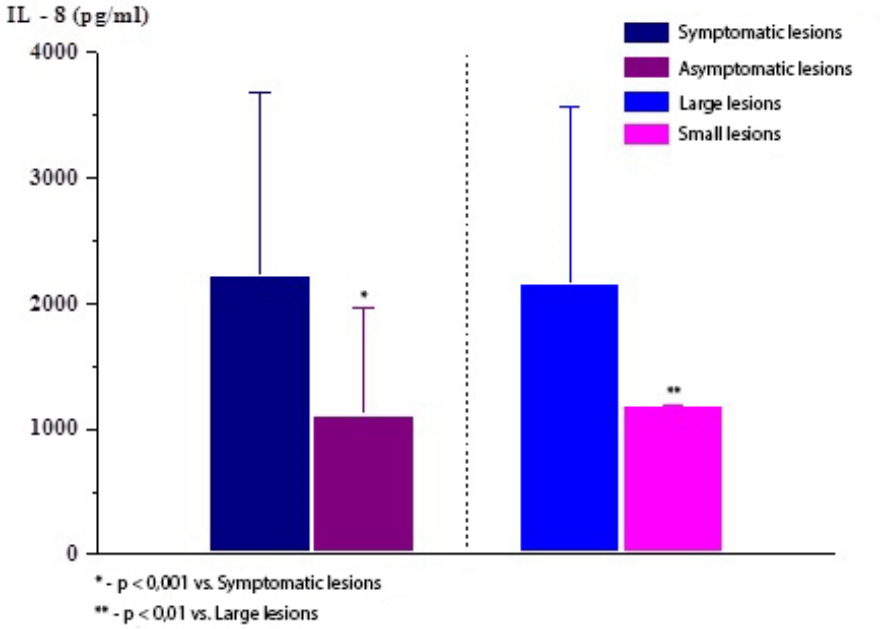

Figure 1. *IL-8 concentrations in relation to clinical symptoms and size of the lesions*

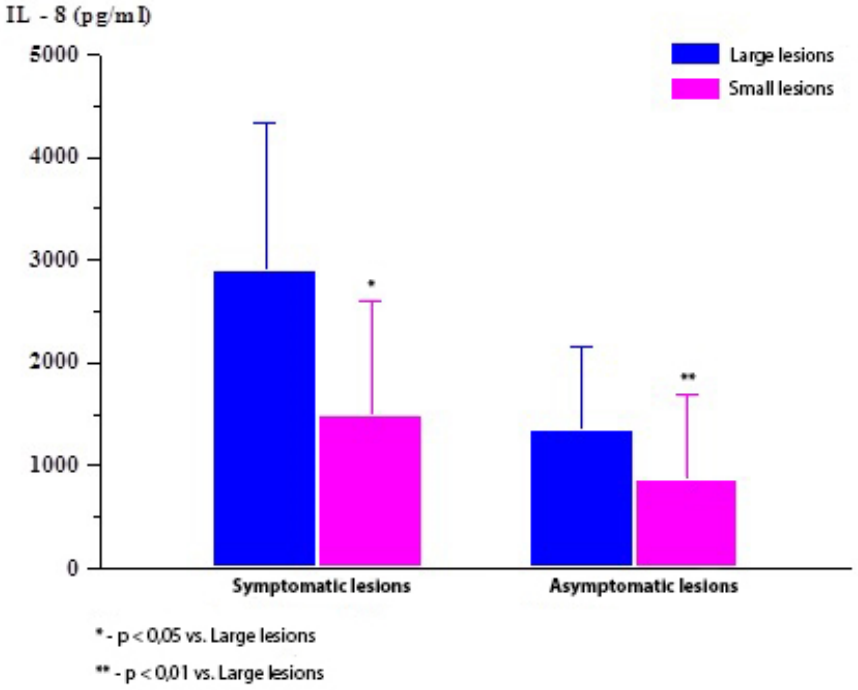

Figure 2. *IL-8 concentrations in individual groups of symptomatic and asymptomatic lesions*

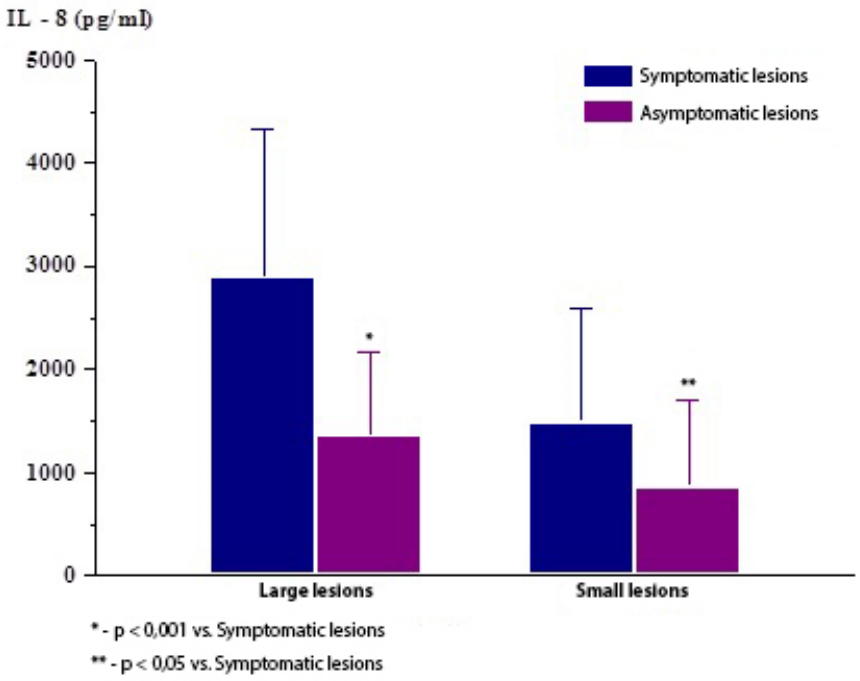

Figure 3. *IL-8 concentrations in individual groups of large and small lesions*

small lesions, it was 1493.79 pg/ml. Large symptomatic lesions had significantly greater concentrations of IL-8 ($p < 0.01$), according to statistical analysis. There was a statistically significant difference in IL-8 concentrations compared to lesion size even in the group of asymptomatic lesions. IL-8 concentrations in asymptomatic large lesions averaged 1356.76 pg/ml, whereas the IL-8 concentrations in asymptomatic small lesions were 870.11 pg/ml ($p < 0.05$).

The concentration of IL-8 within distinct groups of large and small lesions is displayed in Figure 3, where the statistical significance of the data was examined in relation to clinical symptoms. Large symptomatic lesions had a significantly higher concentration of IL-8 (2905.13 pg/ml) than large asymptomatic lesions (1356.76 pg/ml) according to statistical analysis ($p < 0.001$). Additionally, a statistically significant difference was found in small lesions, with the concentration of IL-8 in small symptomatic lesions being 1493.79 pg/ml and 870.11 pg/ml in small asymptomatic lesions ($p < 0.05$).

DISCUSSION

The dynamic balance between proinflammatory cytokines that stimulate inflammation and activate bone resorption and immunoregulatory cytokines that are responsible for suppressing inflammation and healing processes is responsible for the specific pathogenesis of chronic periapical lesions. Inflammatory cells represent an important factor in this process. In the research of Čolić et al. (6, 7), the authors examined the relation of cytokine production to the clinical features of lesions and the organization of infiltrating cells. It was observed that symptomatic lesions contained a high amount of neutrophilic granulocytes. The repeated infection of the root canal cause recruitment of granulocytes into the lesion which is followed by further reactivation of the chronic periapical lesions (4, 8). Granulocytes, as well as the mast cells and macrophages, are important components of inflammatory infiltrates in chronic apical periodontitis and are related to the expression of inflammatory mediators (9). Differentiation of macrophages is show even if lesions are stable or in progression, while prolonged bacterial invasion is able to control the function and differentiation of macrophages associated with development of periapical lesions (10). In response to stimulation by bacterial endotoxins and lipopolysaccharides, periodontal ligament cells also show a high capacity for cytokine and chemokine production, which makes them a crucial factor in the oral innate immunity (11). Other types of stimuli that are not primarily of infectious origin can also increase cytokine production. Data from the literature show that thermal stress can influence gene expression of IL-6 and IL-8 in human periodontal ligament cells, thus proving that these cells can respond to various stimuli with increased cytokine production (12).

IL-8 belongs to the family of chemotaxic cytokines. Chemokines represent a small group of cytokines or proteins that have the ability to induce direct chemotaxis in the nearby specific cells (13). Their main role is to act as chemoattractants and to cause cell migration. One group of chemokines is considered proinflammatory and they are produced during the immune response when they have the role of directing immune cells to the site of infection. One of the first cytokines produced at the area of inflammation is IL-8 and it shows a powerful chemotaxic activity for granulocytes as well as other inflammatory cells (14). Another group of chemokines acts homeostatically and is involved in controlling cell migration during the normal process of tissue maintenance or development. In case of chronic apical periodontitis, chemokines appear to be important elements of a cascade that increases the intensity of localised inflammation, and lead to damage of local tissue and release of mediators that compromise the general health of the host (15).

An increased production of IL-8 in periodontal diseases and gingivitis are presented in literature, and its level correlated with the severity of inflammation (16). High levels of salivary IL-8 were found in patients with developed marginal periodontitis (17). However, it has been proven that inflammatory cytokines and chemokines in case of that pathology originate from the gingival crevicular fluid and not from the salivary glands (18).

IL-8 can activate and recruit osteoclasts at the area of infection, and that can lead to bone resorption in the periapical region and formation of abscess (19). According to Altaie et al. (20), a notable increase in IL-8 gene expression was found in periapical abscesses compared to controls. High values of IL-8 were determined in exudates taken from infected root canals of teeth diagnosed with periapical lesions, thus proving its key role in the acute phase of endodontic disease (19). Reactivation of inflammatory processes can also occur under the influence of IL-17, which, by stimulating the production of IL-8,

leads to exacerbation of chronic apical periodontitis (9). Furthermore, IL-8 was determined in periapical lesions by immunohistochemistry (15). In the study by Lukić et al. (3), mononuclear cells were isolated from periapical lesions, and their ability of IL-8 production was demonstrated. The symptomatic lesions in their study were characterized by a higher infiltratation of granulocytes, which indicated higher levels of IL-8, as well as recent reinfection and disruption of the established chronicity of the inflammatory periapical lesion. Furthermore, IL-8 can activate the release of lysosomal enzymes, generation of superoxide anions, and the expression of adhesion molecules on neutrophils (5). Neutrophil activation may induce tissue damage that causes the worsening of patient symptoms (3).

Data from the literature show that the IL-8 level in periapical lesions correlates with pain in patients with apical periodontitis (21). This statement is in accordance with the results of this study, where a significantly higher concentration of IL-8 was found in the group of symptomatic lesions compared to asymptomatic ones. A statistically significant difference in this research was also demonstrated in the group of large lesions compared to small ones, which proves that IL-8, along with other proinflammatory cytokines, plays a significant role in the contribution to the severity and duration of chronic inflammation.

Inflammatory reactions in patients with apical periodontitis are not limited to the periapical area. Bacteriemias related to apical periodontitis can adversely affect systemic health, especially in patients with compromised general health, cardiovascular disease, pregnancy, or diabetes (2). Patients with compromised health may also have a reduced ability to heal as well as a greater possibility of worsening the outcome of endodontic treatments (22). Blood analyzes in patients after endodontic treatment of teeth with apical periodontitis may show the transient presence of bacteria originating from the root canal of the tooth (23). Cytokines that are locally produced in periapical lesions can be carried by blood to remote parts of the body and cause increased body temperature, sedimentation of erythrocytes, and changes in serum proteins by hepatocytes. In study by Sebring et al. (24), primary apical periodontitis was related to high levels of IL-8 in peripheral blood. Data from the literature show that certain streptococci can activate the production of IL-8 in the human peripheral blood monocytes. Kim et al. (21) concluded that the lipoproteins of *Streptococcus gordonii*, which is isolated in root canal biofilm and periapical lesions and is an important pathogen that can cause infective endocarditis, play a significant role in the production of IL-8 in the human cells of periodontal ligament. Some authors evaluated cytokine levels in the blood of patients before and after surgical removal of periapical lesions. Bakhsh et al. (25) monitored the levels of IL-1β, IL-6, IL-8, TNFα and other mediators in the blood of patients up to one year after periapical surgery and proved that successful endodontic treatment combined with surgical removal of the lesion results in prolonged reduction of the level of inflammatory mediators.

CONCLUSION

It was established that IL-8 was present in every lesion examined. The group with symptomatic lesions had a significantly higher concentration of IL-8 than the group without symptoms, and the group with larger lesions had a significantly higher concentration than the group with smaller lesions. This indicates that IL-8, along with other proinflammatory cytokines, plays a significant role in the development of inflammation and progression of periapical lesions.

Acknowledgment

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Uloga interleukina-8 u razvoju i kliničkoj progresiji hroničnih periapeksnih lezija

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S A Ž E T A K

Uvod/cilj. Hronični apeksni parodontitisi predstavljaju dinamičku reakciju na prisustvo endodontske infekcije u sistemu kanala korena zuba, kada dolazi do aktivacije urođenog i stečenog imunskog odgovora i regrutovanja različitih ćelija i inflamatornih medijatora koji će izazvati posledičnu destrukciju periapeksnih tkiva i formiranje periapeksne lezije. Cilj studije bio je da se odredi koncentracija interleukina (IL-8) u homogenatima tkiva periapeksnih lezija i da se dobijeni rezultati uporede sa simptomatologijom bolesnika i veličinom lezije.

Metode. U studiji su analizirana 93 uzorka tkiva hroničnih periapeksnih lezija. Uzorci su prema kliničkoj simptomatologiji bili podeljeni na simptomatske i asimptomatske, a u odnosu na veličinu na velike i male lezije. Koncentracija IL-8 analizirana je pomoću ELISA testa.

Rezultati. Rezultati su pokazali statistički značajno veću koncentraciju IL-8 u simptomatskim periapeksnim lezijama u poređenju sa asimptomatskim ($p < 0,001$). Koncentracija ovog hemokina takođe je bila statistički značajno veća u velikim lezijama u poređenju sa malim ($p < 0,001$).

Zaključak. Veća koncentracija IL-8 u lezijama sa izraženom kliničkom simptomatologijom, kao i u velikim lezijama pokazuje da je IL-8 važan hemokin koji doprinosi razvoju inflamacije i kliničkoj progresiji periapeksnih lezija.

Ključne reči: **periapeksne lezije, citokini, hemokini, interleukin**