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Original article

Concentration of Transforming Growth Factor-beta 1 in Chronic Periapical Lesions

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SUMMARY

Host response to antigen stimulation in chronic inflammatory periapical lesions is mainly controlled by the balance between proinflammatory and anti-inflammatory cytokines. The aim of this study was to determine the concentration of TGF-β1 in the tissue homogenates of periapical lesions and to analyse its level in relation to the symptomatology of the patients and size of the lesions. Ninety three samples of chronic periapical lesions were obtained after extraction of teeth. Samples were divided according to the clinical symptoms as symptomatic and asymptomatic, and according to the size as large and small. The concentration of TGF-β1 was analyzed using ELISA. The results showed increased production of TGF-β1 in symptomatic lesions compared to asymptomatic, but the difference was not statistically significant. Statistically significant difference in TGF-β1 concentrations was observed in large lesions compared to small (p<0,001). It seems that TGF-β1 have a modulating effect on bone tissue resorption activity under the influence of proinflammatory cytokines and can be molecular prognostic marker of periapical lesion healing.

Key words: periapical lesions, cytokines, TGF-β1

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INTRODUCTION

Dental periapical inflammatory lesions occur as an immune response to chronic stimulation of periapical tissue by infectious agents, bacteria from the root canal and bacterial toxins in the periapical region (1). These lesions are histologically characterized by the formation of fibrous, proliferative epithelium and granulation tissue infiltrated with variety of inflammatory cells (2). The activation of immunocompetent cells leads to the expression of proinflammatory cytokines with subsequent bone tissue resorption and tissue damage in the periapical region (3).

Host response to antigen stimulation in chronic inflammatory periapical lesions is mainly controlled by the balance between proinflammatory and anti-inflammatory cytokines. Transforming growth factor beta (TGF-β) is the anti-inflammatory cytokine which is an important regulator of cell growth, differentiation, inflammation and reparation. TGF-β_1 is of particular importance in the regulation of inflammation and expresses mainly immunosuppressive effects (4). Microbial products, host response to antigens, as well as tissue injuries stimulates the production of TGF-β_1. This cytokine is produced by leukocytes, macrophages, eosinophils, fibroblasts, osteoblasts and osteoclasts (5). TGF-β_1 rapidly evolves multifocal inflammatory response that is characterized by a dense infiltration of lymphocytes and macrophages. However, its suppressive effect can inhibit the proliferation of these cells and to prevent the production of reactive oxygen and nitrogen (6). TGF-β_1 continues to show a strong suppressive effect on proliferation and differentiation of T and B lymphocytes, which further inhibits the production and biological activities of proinflammatory cytokines. Given that it accelerates the healing of soft and hard tissues, it may participate in the reparation of periapical tissue (7). TGF-β_1 also inhibits the formation of osteoclasts. Results of numerous studies suggest that TGF-β_1 is a key mediator of immune homeostasis, including responses in the pulp and periapical region (8, 9).

The aim of this study was to determine the concentration of TGF-β_1 in the tissue homogenates of periapical lesions and to analyse its level in relation to the symptomatology of the patients and the size of the lesions.

MATERIAL AND METHODS

The study involved 93 patients from the Clinic of Dentistry, Niš, who had diagnosed chronic periapical lesions using clinical and radiographic methods. The study was approved by the Ethical Committee of the Faculty of Medicine, University of Niš, Serbia (no. 01-2066-5). Periapical lesions were collected from teeth that were determined as non-treatable and indicated for extraction. Other inclusion criteria were healthy patients not suffering from acute or chronic diseases that could lead to immunodeficiency, who did not take antibiotics and anti-inflammatory medications in previous two months. Only teeth with periapical lesions which did not show moderate or severe form of marginal periodontitis were included in the study. According to subjective symptoms of the patients, lesions were divided into two groups - symptomatic and asymptomatic. Clinically symptomatic lesions were characterized by swelling, pain, discomfort when chewing or sensitivity to percussion and palpation, whereas asymptomatic lesions had no symptoms. The size of periapical lesions was measured in millimeters using a ruler and divided into two groups: small (≤5 mm) and large (≥6 mm) (Table 1). Since periapical lesions contain granulomatous inflammatory tissue that replaces normal bone, there was no equivalent tissue that could be used as negative control. Before administering local anesthetics, teeth, gingiva and mucosa around the tooth were cleaned using 0.12% chlorhexidine and a patient rinsed mouth with 0.12% chlorhexidine for 30 seconds. Samples of periapical lesions removed from the root apex were collected immediately after the extraction using a sterile scalpel, then were washed in sterile saline solution, dried using sterile cotton, placed in a sterile plastic Eppendorf tubes and frozen at -70°C. Using teflon crusher in an iced phosphate buffer at pH 7.4 samples were homogenized with volume adapted to weight of the tissue obtaining the final concentration of 10%. Larger debris was sedimented by centrifugation at 1400 rpm for 1 minute at -40°C. The supernatant was frozen at -70°C until further analysis was performed.

Table 1. ***Periapical lesions according to symptomatology and size***

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

The concentration of TNF-α was measured using ELISA test (R&D Systems Inc. Minneapolis, USA) according to the manufacturer's instructions. The sensitivity of ELISA test for TGF-β_1 was from 1.7 to 15.4 pg/ml, and the concentration of cytokines was analyzed in relation to the size and symptomatology of periapical lesions. Statistical analysis was performed using the Mann-Whitney Rank Sum test using software Sigmastat and Origin. The results were expressed as mean ± standard deviation. P<0.05 was considered statistically significant.

RESULTS

TGF-β_1 was present in all analysed periapical lesions and the examination showed significant results. Figure 1 shows the concentration of TGF-β_1 in all samples, and the average values were analysed with respect to the size and symptomatology. In the group of symptomatic lesions the average concentration was 1458.48 pg/ml, while in the group of asymptomatic lesions the average value was 1287.39 pg/ml. Statistically significant difference in the concentration of TGF-β_1 between symptomatic and asymptomatic lesions was not observed. In the group of large lesions the average TGF-β_1 concentration was 1836.40 pg/ml, whereas in the group of small lesions the average value was 853,68 pg/ml. There was a statistically significant difference between TGF-β_1 concentrations in large and small periapical lesions (p<0.001).

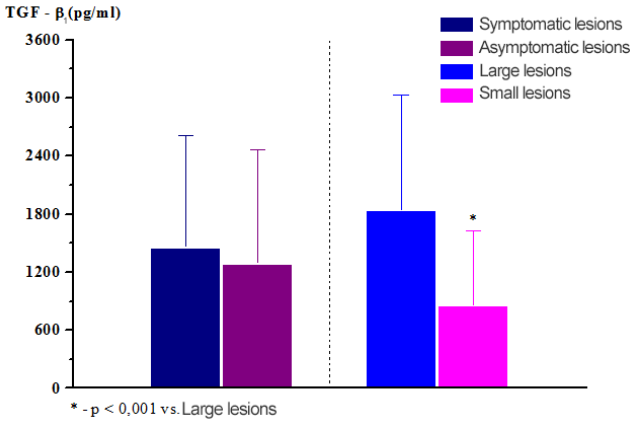

Figure 1. ***TGF-β_1 concentration in tissue homogenates of peripaical lesions in relation to symptomatology and size***

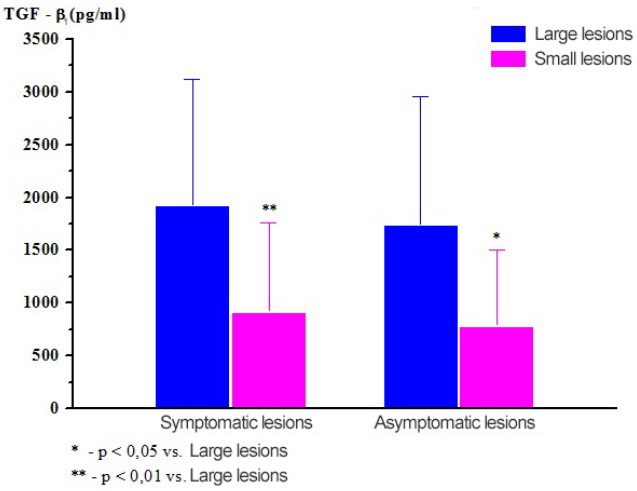

Figure 2. ***TGF-β_1 concentration of symptomatic and asymptomatic periapical lesions***

Figure 2 shows the mean values of TGF-β_1 within the groups of symptomatic and asymptomatic lesions where the average concentrations of TGF-β_1 were analysed with respect to the size of the lesions. The average concentration of TGF-β_1 in symptomatic large lesions was 1926.59 pg/ml, while in symptomatic small lesions it was 920.16 pg/ml. The analysis of the average values showed significantly higher concentrations of TGF-β_1 in symptomatic large lesions

($p<0.05$). Also, in the group of asymptomatic lesions there was statistically significant difference in the concentrations of TGF-β_1 in relation to the size of lesions. The average concentrations of TGF-β_1 in asymptomatic large lesions was 1742.12 pg/ml, whereas it amounted to 787.19 pg/ml in asymptomatic small lesions ($p<0.01$).

Table 2. ***TGF-β_1 concentration of large and small periparical lesions***

Large lesions	n	Middle value ± SD	Mediana	Min.-Max.
Symptomatic lesions	23	1926.59±1197.50	1848.11	128.26-4973.84
Asymptomatic lesions	23	1742.12±1218.41	1744.43	104.40-3914.36
Small lesions				
Symptomatic lesions	23	920.16±836.68	570.88	104.40-2441.12
Asymptomatic lesions	24	787.19±712.71	798.71	107.92-2886.86

Table 2 shows the concentration of TGF-β_1 within the groups of large and small lesions where statistical significance was analysed in relation to the symptomatology. The average concentration of TGF-β_1 in the large symptomatic lesions was 1926.59 pg/ml, while it was 1742.12 pg/ml in large asymptomatic lesions, and the difference was not statistically significant. Statistically significant difference was also not observed in the concentration of TGF-β_1 in small symptomatic lesions (920.16 pg/ml), compared to small asymptomatic lesions (787.19 pg/ml).

DISCUSSION

Cytokine network plays an important role in specific and non-specific immune responses. Many studies have established the production of cytokines in periapical lesions at the level of gene expression, tissue homogenates or cell cultures, and found that in certain circumstances the balance between proinflammatory and immunoregulatory cytokines is disrupted (3, 4). While proinflammatory cytokines, such as IL-1, IL-6, TNF-α, TNF-β, chemokines and Th1 cytokines, propagate inflammation in the periapical tissues and activate osteoclastic bone resorption (6, 7), the role of anti-inflammatory cytokines is important for the suppression of inflammatory processes and healing of the periapical lesions (4, 10, 11).

The study by Gazivoda et al. (3) showed that inflammatory cells from the periapical lesions produced significant levels of proinflammatory (IL-1β, IL-6, IL-8 and TNF-α) and immunoregulatory (IL-10 and TGF-β) cytokines in vitro. The authors examined whether the cytokine production is associated with clinical characteristics and lesion composition of infiltrating cells. In accordance with previous results (11, 12), it was observed that symptomatic lesions contain a higher percentage of neutrophils. The recruitment of granulocytes into the lesion is probably caused by reinfection of the root canal and further reactivation of chronic periapical processes (6, 7). Granulocytes, along with the activated and infiltrating macrophages produce a number of soluble mediators, including the proinflammatory cytokines (13). Under normal circumstances, proinflammatory mechanisms must be strictly controlled to prevent excessive tissue destruction and prevent autoimmune processes. TGF-β and IL-10 are important immunoregulatory cytokines that are produced in the periapical lesions (4, 14, 15). The results of Gazivoda et al. (3) showed that the concentrations of TGF-β and IL-10 in cultures of inflammatory cells did not differ significantly between symptomatic and asymptomatic lesions, suggesting that the anti-inflammatory processes are controlled equally, despite the presence or absence of clinical symptoms.

Bacterial components, particularly lipopolysaccharide, can directly stimulate osteoclast bone resorption, but are relatively weak in strength (4). However, proinflammatory cytokines, originating mainly from immune cells, are of great importance in bone resorption. They stimulate the production and activity of osteoclasts and inhibit the activity of osteoblasts. Production of osteoclasts can be inhibited by a certain anti-inflammatory cytokines, such as TGFβ (16). TGF-β1 is a mediator of wound healing, and is one of the cytokines which regulate local bone remodeling (7). It is produced not only by macrophages, eosinophils, and fibroblasts, but also by osteoclasts and osteoblasts (4). It accelerates the repair of periapical bone loss, and was detected in periapical granulomas and cysts (5, 17). In the study of Danin et al. (4) activity of TGF-β1 has been demonstrated in all granulomas and cysts, but not in the lesions of scar tissue, suggesting that TGF-β1 secreted in the absence of inflammatory response to bacterial components. Thus, TGF-β1 may be a molecular prognostic marker of periapical lesion healing.

In this study, the size of periapical lesions is correlated with the levels of TGF-β1 as a significantly greater amount of TGF-β1 was observed in tissues of large lesions compared to small, whereas in symptomatic lesions amounts of this cytokine was slightly higher than in the asymptomatic. Similar results were obtained by Danin et al. (4) who showed that the amounts of TGF-β1 per milligram of tissue were significantly increased with the size of the lesions.

It cannot be said with certainty what the exact reason for the increased concentration of TGF-β1 in large periapical lesions is. It is not entirely clear whether this is a result of the presence of an increased number of TGF-β1 producing cells or consequence of an increased stimulation of cells with the size of the lesion (4). Gazivoda et al. (3) did not find any significant secretion of TGF-β in cultures of inflammatory cells in large lesions is. One of the reasons may be that the inflammatory cells are not the only one that produce this cytokine, since TGF-β1 is secreted by many cells, including fibroblasts, osteoblasts and osteoclasts. Their research showed that the immunosuppressive mechanisms are much more effective at a later stage of periapical lesion development. To examine whether the immunoregulatory mechanisms in periapical lesions functon *in vitro*, they added exogenous IL-10 and TGF-β to the cultures of inflammatory cells and measured the production of proinflammatory cytokines. They have shown that TGF-β inhibits the production of IL-1β, TNF-α, IL-6 and IL-8 by inflammatory cells isolated from symptomatic and asymptomatic lesions, which confirms its powerful anti-inflammatory activity (18). Their results are consistent with the negative correlation between TGF-β and all of these four proinflammatory cytokines. It is believed that TGF-β has a modulating effect on the bone resorption activity under the influence of proinflammatory cytokines (4).

CONCLUSION

Large lesions showed significantly increased production of TGF-β1 in comparison to small ones, whereas symptomatic lesions had a slightly higher concentration compared to asymptomatic lesions. It seems that TGF-β1 have modulating effect on bone tissue resorption activity under the influence of proinflammatory cytokines, and can be molecular prognostic marker of periapical lesion healing.

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Ispitivanje koncentracije transformišućeg faktora rasta beta 1 u hroničnim periapeksnim lezijama

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SAŽETAK

Balans između proinflamatornih i antiinflamatornih citokina u velikoj meri kontroliše odgovore domaćina na antigenu stimulaciju kod hroničnih inflamatornih periapeksnih lezija. Cilj istraživanja bio je da se odredi koncentracija TGF-β1 u homogenatima tkiva periapeksnih lezija i da se rezultati uporede u odnosu na simptomatologiju pacijenata i veličinu lezije. Ispitivano je 93 uzorka hroničnih periapeksnih lezija dobijenih nakon ekstrakcije zuba. Uzorci lezija su podeljeni prema simptomatologiji pacijenata na simptomatske i asimptomatske, a prema veličini na velike i male. Koncentracija TGF-β1 je ispitivana pomoću ELISA testa, a dobijene vrednosti su analizirane u odnosu na grupe. Rezultati su pokazali povećanu produkciju TGF-β1 u simptomatskim lezijama u odnosu na asimptomatske, međutim, razlika nije bila statistički značajna. Statistički značajna razlika u koncentraciji TGF-β1 uočena je u grupi velikih lezija u poređenju sa malim (p<0,001). Čini se da TGF-β1 ima modulirajući efekat na aktivnost resorpcije koštanog tkiva pod uticajem proinflamatornih citokina i može se smatrati molekularnim prognostičkim markerom zarastanja periapeksnih lezija.

Ključne reči: **periapeksne lezije, citokini, TGF-β1**