SHORT COMMUNICATIONS

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Interleukin 1-beta analysis in chronically inflamed and healthy human dental pulp

Analiza interleukina 1-beta u hronično zapaljenoj i zdravoj zubnoj pulpi

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Abstract

Background/Aim. Proinflammatory cytokines can act like endogenous pyrogen interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF α) which regulate the synthesis of secondary mediators and other proinflammatory cytokines through macrophages and mesenchymal cells. They stimulate acute-phase proteins and attract inflammatory cells. The aim of this study was to determine interleukin 1-β (IL-1 β) concentrations in chronically inflamed and healthy dental pulps. **Methods.** A total of 41 pulps (19 from patients with *pulpitis chronic causa* and 22 from patients with *pulpatis chronic aperta*), divided into two groups, were obtained from teeth with chronic pulp inflammation. The control group consisted of 12 teeth with healthy pulp. After extirpation, pulp samples were immediately placed in sterile Eppendorf tubes and frozen. After that, homogenisation was performed by a Teflon® pestle in ice-cold phosphate buffer solution at pH 7.4 whose volume was adjusted according to the weight of tissue. The supernatant was then frozen at -70°C until the performance of appropriate biochemical analyses. Cytokine IL-1 β value was determined by a commercial enzyme-linked immunosorbent assay (ELISA test). We applied the high sensitivity system technique, which may register low levels of cytokines, ranging from 0.125 to 8.0 pg/mL for IL-1 β. **Results**. By comparing the mean value of IL-1β, in the pulps we can see a statistically significant difference ($p < 0.01$) among them. The highest value of IL-1 β was in the subjects with *pulpitis chronica clausa* and it was 6.21 ± 2.70 pg/mL. **Conclusion**. Proinflammatory cytokine IL-1 β is present in detectable quantities in the pulp tissue of all vital pulps. Its highest concentrations were found in the sample group with *pulpitis chronica clausa.*

Key words:
dental pulp diseases; chronic disease; inflammation; interleukin-1 beta.

Apstrakt

Uvod/Cilj. Proinflamatorni citokin, interleukin 1 (IL-1), interleukin 6 (IL-6) i tumor nekrozni faktor alfa (TNF α) mogu delovati poput endogenih pirogena koji regulišu sintezu sekundarnih medijatora i ostalih proinflamatornih citokina preko makrofaga i mezanhimskih ćelija. Oni stimulišu proteine akutne faze ili privlače ćelije inflamacije. Cilj ove studije bio je da se utvrdi koncentracija interleukina 1-beta (IL-1 β) u hronično zapaljenoj i zdravoj zubnoj pulpi. **Metode.** Ukupno 41 pulpa, podeljena u dve grupe (19 od pacijenata sa *pulpitis chronica clausa* i 22 od pacijenata sa *pulpitis chronica aperta*), dobijene su od zuba sa hroničnim oboljenjem pulpe. Kontrolnu grupu činilo je 12 zdravih pulpi. Uzorci pulpi odmah po ekstirpaciji stavljani su u sterilne ependorf epruvete i zamrzavani. Zatim je vršena homogenizacija telefonskim tučkom u ledenom fosfatnom puferu pH 7,4 čija je zapremina bila prilagođena težini tkiva. Supernatant je nakon toga smrznut na -70°C do izvođenja odgovarajućih biohemijskih analiza. Citokin IL-1β određivan je komercijalnom metodom sendvič enzim-imunoesej tehnikom (ELISA test). Primenjena je tehnika sistema visoke oseljivosti, kojim se mogu registrovati niske koncentracije citokina, u opsegu 0,125–8,0 pg/mL za IL-1β. **Rezultati.** Poređenjem srednjih vrednosti IL-1β (pg/mL), u pulpama uočena je statistički značajna razlika ($p < 0,01$) među njima. Najviše vrednosti Il-1β bile su kod ispitanika sa *Pulpitis chronic clausa* i iznosila je 6,21 ± 2,70 pg/mL. **Zaključak.** Proinflamatorni citokin IL-1β prisutan je u primetnim količinama u pulpnom tkivu kod svih vitalnih pulpi. Njegove najviše koncentracije nađene su u uzorcima zubne pulpe ispitanika sa *pulpitisa chronic clausa.*

Ključne reči:
zub, bolesti pulpe; hronična bolest; zapaljenje; interleukin-1 beta.

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Introduction

Inflammatory and immune system reactions within dental pulp occur as a response to microorganisms and their products which penetrate dentinal tubules [1, 2]. *Pulpitis* is an inflammatory disease of the pulp which is characterised by the local accumulation of inflammatory mediators, including cytokines and chemokines [3]. Depending on the nature of pulp changes and whether the process takes place in the open or closed pulp cavity, we can make a difference between ulcerative and hyperplastic *pulpitis*. Chronic open *pulpitis* is characterised by local vasodilatation and infiltration of mononuclear leukocytes, exudation and cellular infiltration by neutrophil leukocytes. At the pathohistological level we can find granulation tissue present in hyperplastic *pulpitis* which is rich in capillaries and cellular infiltration [4]. Initial infiltration is composed of lymphocytes, macrophages and plasma cells [1]. We can make a difference between the two forms of chronic closed *pulpitis – pulpitis clausa alternativa seu parenchymatosa* and *granulomatosa interna*.

Closed *pulpitis* is characterised by cellular infiltration of small round cells and we can also find degenerative changes of different intensity and nature. Pathohistological examination of internal granuloma indicates the presence of granulation tissue which is rich in vascularised infiltrated round cells which are covered by odontoclasts [4].

The inflamed area is swarmed by inflammatory cells, neutrophils, neutrophil leukocytes, monocytes, macrophages, lymphocytes and plasma cells [5]. Apart from the local accumulation of inflammatory mediators, *pulpitis* is characterised by the presence of cytokines and chemokines [3]. Inflammation degree assessment is a diagnostic problem influencing the decision on what therapy should be applied. However there are no objective, quantitative and clinically practical methods to assess the inflammation degree.

Synchronisation of immune and inflammation reaction depends on the communication between cells through soluble molecules which are generically called cytokines. They include: chemokines, interleukins (IL), growth factors and interferons (IFN) [6]. Interleukins are signal substances which transmit information between different types of leukocytes [7]. Interleunkin-1 beta (IL-1β) is responsible for numerous activities and mediations in host inflammatory response. It is dominantly produced by monocytes/macrophages, fibroblasts, bone cells, endothelial cells, keratinocyte, astrocytes, lymphocytes B, activated lymphocytes T, smooth muscle cells, microglial cells and dendritic cells [8, 9].

Its induction may be caused by microorganisms, microbiological products, inflammatory agents, and antibodies. It is present in pulp tissue and cell culture, which makes it one of the most important interleukins in the inflammatory process occurring in the pulp of teeth. Its main role at low concentrations is to mediate in the local inflammatory process. At high concentrations it has endocrine effects [10]. IL-1 local effects imply the increase in leukocyte adhesion to the endothelial wall, lymphocyte stimulation, neutrophil potentiation, activation of prostaglandin and proteolytic enzymes [11].

Numerous studies suggest that cytokines are markers of pulp inflammation, but the assessment of cytokine level is possible only after pulp extirpation. IL-1β is an important mediator of inflammatory response and local response and, apart from that, it participates in numerous cellular activities such as: proliferation, differentiation and apoptosis. This led us to investigate the level of IL-1β in chronically inflamed and healthy dental pulp, in order to investigate a possible connection between IL-1β level and chronic *pulpitis*. An important and potentially useful outcome of this study would be to find out if the proof of the presence and assessment of IL-1 β level can help in making the diagnosis.

Methods

The research was conducted on 41 human dental pulps with chronic *pulpitis*. The control group consisted of 12 healthy human teeth, which had their pulps extirpated because of prosthetics reasons. After the diagnosis was established on the basis of anamnestic data and basic and auxiliary diagnostic methods, tested teeth were divided into three groups, including the control group. The group 1 (n = 19) consisted of dental pulps with the diagnosed *pulpitis chronica clausa* (*granulomatosa internum s. granuloma internum, alterativa s. parenchymatosa*); The group 2 (n = 22) consisted of dental pulps with the diagnosed *pulpitis chronica aperta* (*ulcerosa huperplastica s. polyposa s. granulomatous*); The group 3 (n = 12), the control group, consisted of healthy teeth and encompassed subjects who had their pulps extirpated because of the prosthetics reasons (Table 1).

Table 1

Classification of investigated pulps into groups according to the diagnosis

Diagnosis	n (%)
Pulpitis chronica clausa	19 (35.83)
Pulpitis chronica aperta	22 (41.5)
Control group	12 (22.64)
Total	53 (100)

After making the diagnosis, the teeth of all the groups were processed in the same manner. Vital pulpectomy was performed. Before the start of the procedure, teeth, gingiva and mucous membrane were cleaned in order to get aseptic working conditions. Anaesthesia with 2% lidocaine was administered in the projection of the root tip for the upper jaw teeth, or mandibular for the lower jaw teeth. Aseptic working conditions were provided by a dental dam and aspirator. Pulp chamber trepanation was performed by a round dental burr. After that, the access cavity was prepared and pulp extirpation was done with a device of an appropriate size. After extirpation, pulp samples were immediately placed in marked sterile Eppendorf tubes and frozen. Then, homogenisation was performed by a Teflon® pestle in ice-cold phosphate buffer solution at pH 7.4 whose volume was adjusted according to the weight of tissue, so that the final homogenate concentration was 10%. The supernatant was then frozen at -70°C until the performance of appropriate biochemical analyses.

Determination of IL-1β concentration – enzyme-linked immunosorbent assay (ELISA) test

In order to measure the IL-1β content in dental pulp, we applied the procedure called ELISA test. For ELISA test we used specific commercial systems of kits particular to IL-1β. The kits are produced by R&D Systems Inc. Minneapolis, USA. We applied the high sensitivity system technique, which may register low levels of cytokines, ranging from 0.125 to 8.0 pg/mL for IL-1 β.

The procedure applied in order to quantify tested cytokines is based on the so-called enzyme-linked immunosorbent assay (Quantikine HS ELISA Assay Principle). Sample density value reading during ELISA test was done by the Ascent plate reader (Thermo Labsystems).

Monoclonal antibodies specific for IL-1β are bound to the surface of a microtitre plate. Standards and samples were added to the sample wells and, during incubation, were bound to antibodies. Microtitre plate wells were then washed out by buffer solution thus removing the excess of unbound cytokines. Enzyme-linked polyclonal antibodies specific for particular cytokines were added to the wells. After incubation the excess of antibody-enzyme reagent was removed by rinsing. Addition of substrate causes colour development which is proportionate to the quantity of cytokines bound in the initial reaction. In order to stop colour development we added 2 N sulphuric acid and colour intensity was read on the wavelength of 450 nm and the obtained values were expressed in pg/mL.

For primary data analysis we used descriptive statistics and statistical hypothesis test. Within descriptive statistics we used measures of central tendency (arithmetic mean), measures of the variability of distribution (standard deviation) and relative numbers. In order to test the hypothesis of the significance of the difference of mean values for independent samples we used Student's *t*-test and analysis of variance (ANOVA) with Tukey *posthoc* test. The statistical significance criterion was $p < 0.05$ or $p < 0.01$. Statistical processing of the results was done by a software package used for statistical analysis, SPSS (version 21).

Results

Cytokine concentration in a homogenate of healthy and chronically inflamed dental pulps was determined by the application of ELISA test. Tests encompassed 53 pulp samples (including the control group) and the cytokine concentration was analysed by the following groups: chronically inflamed, open and closed *pulpitis*.

Examination of the IL-1β concentration in pulp tissue showed a significant cytokine concentration in all samples. Figure 1 shows IL-1β concentrations in the patients with chronic *pulpitis* (n = 41) and the control group (n = 12). The arithmetic mean of IL-1β in the patients with chronic *pulpitis* was 4.24 ± 2.64 pg/mL (range, 1.54–8.82 pg/mL), and in the patients from the control group 3.94 ± 1.26 pg/mL (range, 2.5–6.89 pg/mL). There was no statistical difference between the patients with chronic *pulpitis* and the control group related to IL-1β values ($t = 0.543$, $p = 0.590$) (Figure 1).

The arithmetic mean of IL-1β in the patients with chronic closed *pulpitis* was 6.21 ± 2.70 pg/mL (range, 1.71–8.82 pg/mL), in the patients with open *pulpitis* 2.54 ± 0.66 pg/mL (range, 1.54–3.67), and in the patients from the control group 3.94 ± 1.26 pg/mL (range, 2,5–6,89 pg/mL). Among the patients with chronic closed *pulpitis*, chronic open *pulpitis* and the control group there was a statistically significant difference related to IL-1β values ($F = 21.883$, $p < 0.01$).

IL-1β values were statistically significant higher in the group with chronic close *pulpitis* when compared to the group of the patients with chronic open *pulpitis* ($p < 0.01$), and in the group with chronic closed *pulpitis* when compared to the patients from the control group ($p < 0.01$), but the difference was not statistically significant between the patients with chronic open *pulpitis* and the patients from the control group ($p = 0.081$) (Figure 2).

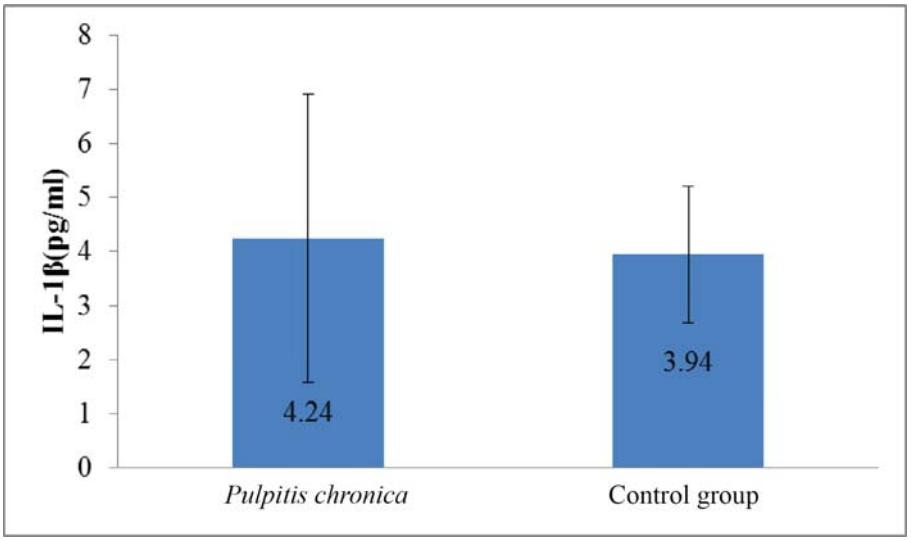

Fig. 1 – Interelukin-1 beta (IL-1β) values in subjects with chronic pulp inflammation and the control group

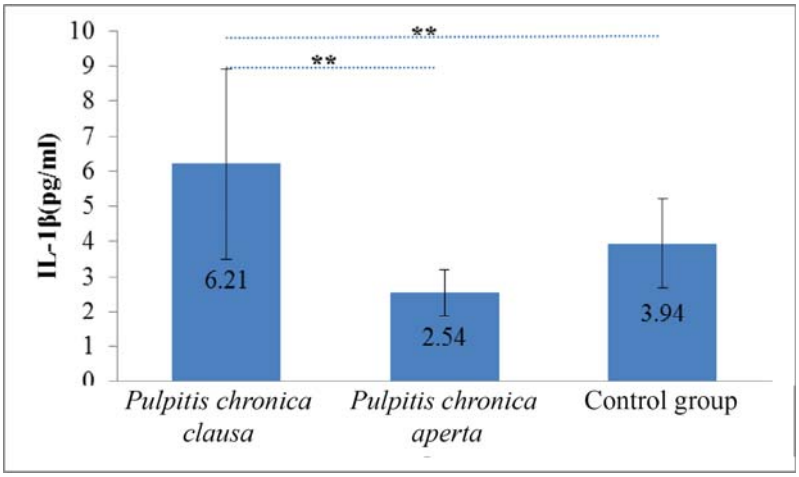

Fig. 2 – Values of Interelukin-1 beta (IL-1β) in the subjects with chronic pulpitis clausa, pulpitis chronica aperta and the control group.
****statistically significant difference ($p < 0.01$).**

Discussion

Biological response of pulp to stimuli and events which control these processes are still not clear [12]. Contemporary methods of molecular biology and genetic engineering make it possible for all laboratories to quantitatively and qualitatively determine cytokines in body fluids or tissue cell cultures. Due to a more frequent use of cytokines as possible modulators of inflammation, immunity and haematopoiesis, they are often called biological response modulators. Several biological molecules [tumor necrosis factor α (TNF α), IL-8, IL-2)] are identified and found in inflamed pulp in higher concentrations than those registered in healthy pulp. These substances attract immune cells such as polymorphonuclear cells and macrophages [12]. Diagnostic assessment of biologic markers may improve validity of predicting or treating inflammatory pulp disease [13]. In our research, we analysed the concentration of proinflammatory cytokine in pulp tissue of chronically inflamed and healthy dental pulp. IL-1 was the first described as a lymphocyte activating factor and it was then discovered that IL-1 has a number of other biological activities and that there are at least two major types of IL-1 (alpha and beta) which bind to the same receptor [14].

Present researches are related to the examination of pulp tissue and cytokine expression in healthy and inflamed pulp. Production of IL-1, IL-2, IL-6, IL-8, IL-18 and TNFα and cyclooxygenase-2 (COX-2) in pulp tissue with symptomatic inflammation was published by numerous authors in their studies [15–21].

After damaging dental pulp, some cells produce cytokines important for initiating and controlling the inflammation process. In this case, the most important cytokines are: IL-1β and IL-8. Silva et al. [10] in their study analysed the location, distribution and concentration of IL-1β and IL-8 in healthy and inflamed dental pulps. Immunohistochemical results confirm that the samples of inflamed pulps were stronger positive for both cytokines than those from healthy pulps. The results of our study do not completely match those from the abovementioned studies [10, 14, 22–24]. The difference in the concentration of IL-1β in chronically inflamed and healthy dental pulp has no statistical significance ($t = 0.543$, $p = 0.590$). However, the difference is statistically significant in the group with chronic close *pulpitis* when compared to the group of the patients with chronic open *pulpitis* ($p < 0.01$), and in the group with chronic closed *pulpitis* when compared to the patients from the control group ($p < 0.01$). Higher level of IL-1β in closed *pulpitis*, in comparison to the control group is expected, which is also in accordance with other studies [10, 15, 25–27], since it is known that IL-1β stimulates the inflammation process. The difference is not statistically significant between the patients with chronic open *pulpitis* and the control group ($p = 0.081$). This level of IL-1β concentration in chronic open *pulpitis* remains somewhat a mystery and poses a question for a new, expanded study. Maybe the reason of the decrease in IL-1β concentration in chronic open *pulpitis* can be linked to the fact that pulp is in an advanced inflammation stage leading to pulp necrosis. In 1985 Hes [28] wrote that chronic open *pulpitis* is always total, meaning that infection and inflammation engulfs the pulp up to the apex. Systematic assessment of the results of the earlier studies, ours included, suggests that gene expressions of cytokines increases in pulp tissue during inflammation. Findings about the important roles of IL-1β, IL-6, IL-17 and TNF-α in pathogenesis of chronic inflammation may be used in finding the most efficient therapy for the treatment of chronic diseases. Herman et al. [29] think that in the future we can expect the development of new medicine which will act as anti-cytokine, thus preventing cytokines to bind with their receptors. In this manner we would be able to selectively and with less side effects block a particular cytokine [29].

Conclusion

On the basis of the obtained data we conclude that IL-1β could be found in all vital pulp tissues. The highest concentrations of this protein were found in the sample group with *pulpitis chronica clausa*.

R E F E R E N C E S

1. *Tahmiščija I, Radović S, Berisalić A, Korać S, Džanković A*. Histolopathological Features of Irreversible Pulpitis. Stomatološki vjesnik 2012; 1: 35−8. (Bosnian)
2. *Freitas P, Novaretti CP, Rodini CO, Batista AC, Lara VS*. Mast cells and lymphocyte subsets in pulps from healthy and carious human teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103(5): e95−102.
3. *Kokkas A, Goulas A, Stavrianos C, Anogianakis G*. The role of cytokines in pulp inflammation. J Biol Regul Homeost Agents 2011; 25(3): 303−11.
4. Filipović V, Gvozdenović-Sedlecki S, Karadžov O, Kezel D, Kolak Ž, Kuburović D, et al. Endodontics. Belgrade: Dečije novine; 1989. (Serbian)
5. *Hahn CL, Liewehr FR*. Update on the adaptive immune responses of the dental pulp. J Endod 2007; 33(7): 773−81. (Serbian)
6. *Šurbatović M, Jovanović K, Vojvodić D, Filipović N, Babić D*. Importance of determination of proinflammatory cytokines in the blood of polytraumatized patients with sepsis. Vojnosanit Pregl 2004; 61(2): 137−43. (Serbian)
7. *Bubanović I, Anđelković Z, Mirić M, Milosavljević Z, Mitić R*. Importance of cytokines in the regulation of the specific immune response Praxis Medica 2004; 32(1−2) 61−5. (Serbian)
8. *Čulić S*. Cytokines and autoimmune diseases. Paediatr Croat 2005; 49(Suppl 1): 148−61. (Croatian)
9. *Koch AE, Kunkel SL, Strieter RM*. Cytokines in rheumatoid arthritis. J Investig Med 1995; 43(1): 28−38.
10. *Silva AC, Faria MR, Fontes A, Campos MS, Cavalcanti BN*. Interleukin-1 beta and interleukin-8 in healthy and inflamed dental pulps. J Appi Oral Sci 2009; 17(5): 527−32.
11. *Nair PN*. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004; 15(6): 348−81.
12. *Rauschemberger CR, Bailey JC, Cootanco CJ*. Detection of human IL-2 in normal and inflamed dental pulps. J Endod 1997; 26(6): 366−70.
13. *Pezelj-Ribaric S, Anic I, Brekalo I, Miletic I, Hasan M, Simunovic-Soskic M*. Detection of tumor necrosis factor alpha in normal and inflamed human dental pulps. Arch Med Res 2002; 33(5): 482−4.
14. *Piattelli A, Trisi P, D'Addona A*. Bone resorption and cytokines: the role of IL-1 beta, TNF and lymphotoxin. Minerva Stomatol 1990; 39(6): 447−51.
15. *D'Souza R, Brown LR, Newland JR, Levy BM, Lachman LB*. Detection and characterization of interleukin-1 in human dental pulps. Arch Oral Biol 1989; 34(5): 307−13.
16. *Huang GT, Potente AP, Kim JW, Chugal N, Zhang X*. Increased interleukin-8 expression in inflamed human dental pulps. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999; 88(2): 214−20.
17. *Lin SK, Kuo MY, Wang JS, Lee JJ, Wang CC, Huang S,* et al. Differential regulation of interleukin-6 and inducible cyclooxygenase gene expression by cytokines through prostaglandin-dependent and –independent mechanisms in human dental pulp fibroblasts. J Endod 2002; 28(3): 197−201.
18. *McLachlan JL, Sloan AJ, Smith AJ, Landini G, Cooper PR*. S100 and cytokine expression in caries. Infect Immun 2004; 72(7): 4102−8.
19. *Nakanishi T, Matsuo T, Ebisu S*. Quantitative analysis of immunoglobulins and inflammatory factors in human pulpal blood from exposed pulps. J Endod 1995; 21: 131−6.
20. *Kokkas AB, Goulas A, Varsamidis K, Mirtsou V, Tziafas D*. Irreversible but not reversible pulpitis is associated with up-regulation of tumour necrosis factor-alpha gene expression in human pulp. Int Endod J 2007; 40(3): 198−203.
21. *Nakanishi T, Shimizu H, Hosokawa Y, Matsuo T*. An immunohistological study on cyclooxygenase-2 in human dental pulp. J Endod 2001; 27(6): 385−88.
22. *Barkhordar RA, Hayashi C, Hussain MZ*. Detection of interleukin-6 in human dental pulp and periapical lesions. Endod Dent Traumatol 1999; 15(1): 26−7.
23. *Guo X, Niu Z, Xiao M, Yue L, Lu H*. Detection of interleukin-8 in exudates from normal and inflamed human dental pulp tissues. Chin J Dent Res 2000; 3(1): 63−6.
24. *Wang D, Ding WS, ZJ, Liu ZH,Wang JH*. Detection of TNF-α in dental pulps of normal, acute and chronic pulpitis. J Shandong Univ 2005; 1: 25.
25. *Barkhordar RA, Ghani P, Russell TR, Hussain MZ*. Interleukin-1ß activity and collagen synthesis in human dental pulp fibroblasts. J Endod 2002; 28(3):157–9.
26. *Hosoya S, Matsushima K*. Stimulation of interleukin-1ß production of human dental pulp cells by porphyromonas endodontalis lipopolysaccharide. J Endod 1997; 23(1): 39–42.
27. *Lertchirakarn V, Birner R, Messer HH*. Effects of interleukin-1 beta on human pulpal fibroblast proliferation and collagen synthesis. J Endod 1998; 24(6): 409–13.
28. *Hess JC*. Enseignement d´ odontologie conservatrice. Paris: Maloine; 1985.
29. *Herman S, Kronke G, Schett G*. Molecular mechanisms of inflammatory bone damage. Emerging targets for therapy. Trends Mol Med 2008: 14(6): 245−53.

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