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

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

Ljiljana Šubarić*, Aleksandar Mićić†, Vladimir Matvijenko*, Radovan Jovanović*, Dušan Živković*, Dejan Perić*, Zoran Vlahović†

*Department of Restorative Dentistry and Endodontics, †Department of Oral Surgery, Clinic of Dental Medicine, Faculty of Medicine, University of Priština/Kosovska Mitrovica, Kosovska Mitrovica, Serbia; ‡Department of Restorative Dentistry and Endodontics, Clinic of Dental Medicine, Faculty of Medicine, University of Niš, Niš, Serbia

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 clausa and 22 from patients with pulpitis 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 extrpation, 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-1 β, 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 chronic clausa and it was 6.21 ± 2.70 pg/mL. Conclusions. 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 chronic 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 nekrozn faktor alfa (TNF α) mogu delovati poput endogenih pirogena koji regulišu sintezu sekundarnih medijatora i ostalih proinflamatornih citokina preko makrofaga i mezenhimalnih celija. Oni stimulisu proteine akutne faze ili privlače ĉelije inflamacije. Čilj 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 pulpitisa chronica clausa i 22 od pacijenata sa pulpitisa chronica aperta), dobijene su od zuba sa hroničnom oboljenjem pulpe. Kontrolnu grupu činilo je 12 zdravih pulpi. Uzorci pulpi odmah po ekstrakciji stavljani su u sterilne eppendorf crvene i zamrzan. Zatim je vršena homogenizacija telefonskim tučkom u ledenom fosfatnom puferu pH 7,4 čija je zapremina bila prilagođena težini tkiva. Supernata-
tant je nakon toga smrznut na -70°C do izvođenja odgovarajućih biohemijijskih analiza. Citokin IL-1 β određen je komercijalnom metodom sendvič enzim-imunoosej tehnikom (ELISA test). Primjena je tehnika sistema visoke osevljivosti, kojim se mogu registrovati niske koncentracije citokina, u opsegu 0,125–8,0 pg/mL za IL-1 β. Rezultati. Poređenjem srednjih vrednosti IL-1 β (pg/mL), u pulpama uočena je statistički značajna razlika (p < 0,01) među njima. Najviše vrednosti IL-1 β bile su kod ispitanika sa pulpitisa chronica clausa i iznosila je 6,21 ± 2,70 pg/mL. Zaključak. Proinflamatorni citokin IL-1 β prisutan je u primetnim količinama u pulpnom tkivu kod svih vitalnih pulpi. Njegove najviše koncentracije nadvje su u uzorcima zubne pulpe ispitanika sa pulpitisa chronica clausa.

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

Inflammatory and immune system reactions within dental pulp occur as a response to microorganisms and their products which penetrate dental tubules. Pulpitis is an inflammatory disease of the pulp which is characterised by the local accumulation of inflammatory mediators, including cytokines and chemokines. 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 pathological level we can find granulation tissue present in hyperplastic pulpitis which is rich in capillaries and cellular infiltration. Initial infiltration is composed of lymphocytes, macrophages and plasma cells. We can make a difference between the two forms of chronic closed pulpitis – pulpitis clausa alterativa 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.

The inflamed area is swarmed by inflammatory cells, neutrophils, neutrophil leukocytes, monocytes, macrophages, lymphocytes and plasma cells. Apart from the local accumulation of inflammatory mediators, pulpitis is characterised by the presence of cytokines and chemokines. 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). Interleukins are signal substances which transmit information between different types of leukocytes. Interleukin-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.

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. IL-1 local effects imply the increase in leukocyte adhesion to the endothelial wall, lymphocyte stimulation, neutrophil potenti-ation, activation of prostaglandin and proteolytic enzymes.

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-1β 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 interna n. granuloma internum, alterativa s. parenchymatosa); The group 2 (n = 22) consisted of dental pulps with the diagnosed pulpitis chronica aperta (ulcerosa hyperplastica 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

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>Pulpitis chronica clausa</td>
<td>19 (35.83)</td>
</tr>
<tr>
<td>Pulpitis chronica aperta</td>
<td>22 (41.5)</td>
</tr>
<tr>
<td>Control group</td>
<td>12 (22.64)</td>
</tr>
<tr>
<td>Total</td>
<td>53 (100)</td>
</tr>
</tbody>
</table>

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-1β.

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 – Interleukin-1 beta (IL-1β) values in subjects with chronic pulp inflammation and the control group](image-url)
Fig. 2 – Values of Interleukin-1 beta (IL-1β) in the subjects with chronic pulpitis clausa, pulpitis chronica aperta and the control group.

**statistically significant difference ($p < 0.01$).
REFERENCES


