Detection and Assessment of Interleukin 6 in Irreversible Pulp Inflammation

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The study aimed to assess the number, localization and distribution of interleukin 6 (IL-6) positive cells in healthy pulp, acute and chronic pulpitis. The study group included 48 patients aged between 18-72, treated in University of Medicine and Pharmacy Grigore T. Popa Iasi, Romania. The pulpectomy was performed on 42 patients diagnosed with acute and chronic pulpitis. The other 6 patients, without signs of dental caries or periodontal disease, were submitted to extractions of teeth for orthodontic purposes, with pulpectomy performed before extraction. The pulp samples were examined with optical microscope. The detection and assessment of IL-6 were performed using immunohistochemical technique. Data were statistically analysed using non-parametric tests. According to morphopathological criteria, 42.85% were classified as acute pulpitis and 57.14% as chronic pulpitis. The pulp samples in control group were not associated with IL-6 positive cells. The analysis of all samples with acute and chronic pulpitis identified 73.80% samples with IL-6 and 26.20% associated with the absence of IL-6. The highest frequency of IL-6 positive cells was recorded in rich-cell zone of crown dental pulp. The systemic distribution of IL-6 positive cells was mostly diffused without well-defined orientation. IL-6 release in acute and chronic pulpitis is significantly higher comparing with healthy pulp tissue.

Keywords: acute pulpitis, chronic pulpitis, cytokine, ekoscope

Dental pulp reacts against repeated exposures to bacteria due to autoimmune specific and nonspecific mechanisms. The pulp contamination take place through various invasion pathways related with dental tubules [1]. The level of pulp damage varies from mild inflammation to pulp necrosis associated with periapical lesions [2]. The researches regarding pulp tissues identified immune cells and cell that have the ability to recognize foreign antigens. The pulp-dental tissue is infiltrated initially with chronic inflammatory cells, like macrophages, lymphocytes and plasmatic cells [3]. The analysis of cytokines represents an essential key to understand the etiopathogenetic reactions of different dental diseases [4]. The cytokines are soluble proteins that play an important role in the initiation and maintaining of inflammatory immune responses as well as in intercellular communications. These cytokines include interleukins (IL): IL-1α, IL-1β, IL-6, IL-8 and necrosis tumor factor (TNF-α). Neutrophils and macrophages are major producers of interleukins; other cells that release interleukins are phagocytes, vascular endothelial cells, and keratinocytes [5]. One of the most important interleukin intervening in oromaxillofacial area is interleukin 6 (IL-6); IL-6 influences the evolution of pulpitis, periapical lesions, chronic marginal periodontitis, gingivitis, odontogenic cysts, lichen planus [6-8]. Interleukin-6 is a multifunctional cytokine with both pro-inflammatory (induces acute phase reactants in liver) and anti-inflammatory (regulates neutrophils function and secretion of pro-inflammatory cytokines) features, as a response to trauma and infectious agents [9-12]. IL-6 is able to stimulate a number of biological processes including antibody production, cells T activation, cells B differentiation, proteins growth in acute phase, hemopoiesis, angiogenesis, vascular permeability, osteoclasts differentiation [13, 14]. IL-6 is produced as a result of interactions between gram negative bacteria, their metabolites (exotoxins, endotoxins) and various inflammation mediators (neuropeptides, kinines, complement system, metabolites), in relation with quantity, time and pathogenicity [9-12, 15].

Within this context, our study aimed to assess the number of IL-6 positive cells, localization and distribution in healthy pulp, acute pulpitis and chronic pulpitis.

Experimental part

The study group included 48 patients, 28 men and 20 women, aged between 18-72 years and the average age was 48 years, treated in Clinical Base of Medical Dental Teaching Mihail Kogalniceanu - University of Medicine and Pharmacy Grigore T. Popa Iasi, Romania. The pulpectomy was performed to 42 patients, diagnosed with acute and chronic pulpitis. The other 6 patients, without signs of dental caries or periodontal disease, were submitted to extractions of teeth for orthodontic purposes, with pulpectomy performed before extraction.

The study has been approved by a research ethics committee of Medicine and Pharmacology University

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Clinical and morphopathological diagnosis

The clinical diagnosis of acute pulpitis was performed by a number of symptoms and signs: (i) symptomatology (spontaneous pain as pulp hypersensitivity), (ii) positive intense response to sensitivity pulp test (prolonged pain, over 8 min, after the removal of cold stimulus), represented by the application of tetrafluoroethane, Pharmaceutal Spray, Septodont®, (iii) pain provoked by tooth percussion. For chronic pulpsitis study group the diagnosis was reported according to the next symptoms and signs: (i) symptomatology (provoked and/or spontaneous pain as pulp hyposensitivity), (ii) mild positive response to pulp sensitivity testing (after the removal of cold stimulus, represented by the application of tetrafluoroethane, Pharmaceutal Spray, Septodont®), (iii) blood and pain after palpation of exposed pulp tissue [16-18].

The testing of pulp sensitivity was performed comparatively with homologous teeth. The criteria of morphopathological diagnosis for acute pulpitis were as follows: vascular congestion, polymorphic inflammatory infiltration, edema, destructive alterations of odontoblastic layer. The criteria of morphopathological diagnosis for chronic pulpitis were as follows: fibrous and sclerotic atrophy, chronic inflammatory infiltrate, hyaline degeneration and cellular detritus [19].

Therapeutic protocole of vital pulp extirpation (pulpectomy)

Irreversibly inflamed dental pulp is removed (by pulpectomy) for curative therapy of this diseases. The pulpectomy was performed accordingly to defined standards: locoregional anesthesia (Usbistesin Forte 4%, 3M ESPE®), operatory field isolation with diaga and salivary aspiration system, tooth antisepsitation with NaOCl 2% (Cerammed®, D). The photos were processed using digital foto device Leica DM 750 (Leica Microsystems GmbH®, D). The samples were classified in relation to number of IL-6 expressing positive IL-6 (colored IHC with brown substrate).

The samples were classified in relation to number of IL-6 expressing positive IL-6: (i) score 0, absence of IL-6, (ii) score 1-IL-6 poor expression IL-6 (<3 positive cells/5 cm²), (iii) score 2-IL-6 mild expression (<6 pro-inflammatory cells/5 cm²), (iv) score 3-high level of IL-6 expression (>6 pro-inflammatory cells/5 cm²). This classification was performed using device Ekoscope (Eon Trading LLC), based on optic microscopy, and used for calculation of somatic cells in complex research applications. The final score for acute and chronic pulpsitis samples was obtained by calculation of mean values compared with morphopathological diagnostic. This method allowed the establishment of clear limits between IL-6 values between acute and chronic pulpsitis study groups.

Statistical analysis

To compare morphopathological diagnostic with IL-6 values between acute and chronic pulpsitis study groups, data were statistically analysed using non-parametric test Mann-Whitney and Kruskal-Wallis, with p=0.05.

Results and discussions

Accordingly to morphopathological criteria, 18 cases (42.85%) were classified as acute pulpsitis and 24 cases (57.14%) as chronic pulpsitis. The pulp samples in control group were not associated with IL-6 expression. In the study group with acute pulpsitis, the scores distribution was as follows: three cases (16.66%) with score 0, for cases (22.22%) with score 1, five cases (27.77%) with score 2 and six cases (33.33%) with score 4 (table 1). In the study group with chronic pulpsitis, the scores distribution was as follows: eight cases (33.33%) with score 0, six cases (25%) with score 1, seven cases (29.16%) with score 2 and three cases (4.16%) with score 4 (table 1).
The analysis of all samples with acute and chronic pulpitis identified 31 pulp samples (73.80%) with cells expressing positive IL-6 and 11 pulp samples (26.20%) associated with the absence of positive IL-6, perhaps due to anti-inflammatory medication.

Regarding location of IL-6 in pulp tissue, the highest frequency was recorded in rich-cell zone of crown dental pulp, perhaps due to the higher number of fibroblasts, macrophages, neutrophils and vascular endothelial cells.

The systemic distribution of IL-6 was mostly diffused without well-defined orientation, excepting seven cases where it was found a cluster distribution (fig. 1).

The specific features of chronic pulpitis samples were as follows: high level of collagen fibers in central pulp area, fibroblastic growth, disordered collagen fibers, chronic inflammatory infiltrate, diffuse calcification around blood vessels (fig. 2 and 3).

**Table 1**

<table>
<thead>
<tr>
<th>Score</th>
<th>Absent marking</th>
<th>Showed weak marking</th>
<th>Average mark</th>
<th>Intense mark</th>
<th>Total</th>
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<tbody>
<tr>
<td>0 Pulpitis chronic acute Total</td>
<td>8</td>
<td>3</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>1 Pulpitis chronic acute Total</td>
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<tr>
<td>2 Pulpitis chronic acute Total</td>
<td>7</td>
<td>5</td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>3 Pulpitis chronic acute Total</td>
<td>3</td>
<td>6</td>
<td></td>
<td></td>
<td>9</td>
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</table>

![Fig. 1. Acute pulpitis (a) with: edema (A) and cell infiltration (B) among conjunctive structures of pulp, mechanical dilaceration and moderate leukocyte diapedesis (Col. HE, x400), respectively acute pulpitis with high level of IL-6 expression (b) (brown surface staining) and cluster arrangement (C) and (D) (IHC, anti IL-6, x400) (IHC, anti IL-6, x400)](image1)

![Fig. 2. Chronic pulpitis (a) with fibroblastic proliferation (A) and disordered arrangement of collagen fibers (B) (Col. HE, x400) and respectively chronic pulpitis (b) with proliferative fibrous, sclerosis, neoformation vessels and with IL-6 absence (C) and (D) (IHC, anti IL-6, x400)](image2)

![Fig. 3. Chronic pulpitis (a) with collagen fibers (A) and fibroblastic proliferation (B) (Col. HE, x400), respectively (b) chronic pulpitis with IL-6 poor expression (brown surface staining) (C) (IHC, anti IL-6, x400)](image3)
In chronic pulpitis samples have been identified the peripheral odontoblastic layer. The specific features of acute pulpitis samples were as follows: vascular congestion, pulp edema, leukocytes exude, cells detritus (fig. 1 and 4).

The recorded data were statistically analysed. The mean rank of acute pulpitis was higher comparing with chronic pulpitis. Test Mann-Whitney (comparing the mean scores values between study groups) did not found the existence of significant statistical differences (U 153.5, p = 0.101 > 0.05) (table 2).

For category score zero, mean values rank of chronic pulpitis is equal to mean values rank of acute pulpitis. Related to samples classified in score zero, there is no significant statistical differences between chronic pulpitis and acute pulpitis (U 12.0, p = 1 > 0.05). For category score one, mean values rank of chronic pulpitis is higher comparing to mean values rank of acute pulpitis. Related to samples classified in score one, were found significant statistical differences between chronic pulpitis and acute pulpitis (U 4.0, p = 0.046 < 0.05). For category score two, the mean values rank of chronic pulpitis is also higher than acute pulpitis. Significant statistical differences were found between chronic pulpitis and acute pulpitis classified as score two (U 4.0, p = 0.017 < 0.05). In score three category, the mean values rank of chronic pulpitis is lower comparing with acute pulpitis, without significant statistical differences (U 5.0, p = 0.258 > 0.05).

Despite high number of studies describing pulp pathology in relation to clinical and therapeutical issues, a few number of studies are focused on the relation between morphopathological diagnostic and presence of IL-6 associated with inflammatory processes in pulp tissue [3,7,15,20-24]. The results of our study, related to localization, distribution, number, presented similar values with those described by literature data. In this context, we must highlight a few relevant aspects regarding the correspondences between histopathological and immunological issues. The release of IL-6 is stimulated by peptidoglycans derived from gram positive bacteria in carious dentine; the concentration of IL-6 released by pulp cells is dependent by action time and peptidoglycans dose [15]. Tokuda M. et al. (2001) demonstrated the stimulation of IL-6 secretion in pulp human cells under the action of lipopolysaccharides Prevotella intermedia. The fibroblasts are implied in the pathogenesis of pulp inflammation through IL-6 production, accordingly to Lin S.K. et al. (2002). The release of excessive IL-6 levels conducts to transformation of acute reversible phase of pulp inflammation in chronic irreversible phase [25]. Elsalhy M. et al. (2013) found significant higher levels of IL-6 in pulp samples of teeth exposed to deep dental caries. The ratio IL-6/IL-10 is significantly higher in pulp tissue with irreversible pulpitis, comparing with pulp tissue derived from teeth with deep dental caries. The author considers that ratio IL-6/IL-10 can be considered a marker of pulp inflammation in samples of pulp tissue derived from teeth with deep dental caries. Also in this study, mean value of IL-6 levels was 36 +/- 3.9 pg/mg protein in samples with chronic pulpitis and only 0.01 +/- 0.02 pg/mg protein in healthy pulp tissue samples. Park H.S. (2002) determined, using ELISA test, levels of IL-6 in dental pulp with chronic pulpitis. The mean level of IL-6 in sample with tissue affected by pulp inflammation was 43.62 pg/mg protein while control group (healthy pulp tissue) had a mean value of 24.41 pg/mg protein. In same study, author found the existence of PMN inflammatory infiltrate in tissue affected by pulp inflammation and the absence of PMN infiltrate in control group. Also Nakaniishi T. et al. (1995) showed the existence of higher levels of IL-6 in samples with pulp inflammation comparing with healthy pulp tissue.

Despite the detection of IL-6 both in incipient and advanced stages of pulp inflammations, the exact role of these cytokines in the pathogenesis and progression of pulp inflammation is not well defined.

Conclusions

The mean values rank of scores for acute pulpitis is higher comparing with chronic pulpitis. For score one with U 4.0, p = 0.046 < 0.05 and score 2 with U 4.0, p = 0.017 < 0.05, values rank in chronic pulpitis is higher comparing with values rank in acute pulpitis. The mean rank for score 3 was lower in chronic pulpitis, comparing with acute pulpitis, without significant statistical differences - U 5.0, p = 0.258 > 0.05. The number of IL-6 positive cells in study group was higher comparing with control group (p < 0.05). Higher frequency of IL-6 was recorded in pulp cell-rich zone of crown dental pulp. The systemic distribution of IL-6 was mostly diffuse without well-defined orientation.

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Table 2

<table>
<thead>
<tr>
<th>TESTS STATISTICSa</th>
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<tr>
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<tr>
<td>Wilcoxon W</td>
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<tr>
<td>Z</td>
<td>-1.642</td>
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<tr>
<td>Asymp.Sig. (2-tailed)</td>
<td>.101</td>
</tr>
</tbody>
</table>

a. Grouping Variable: Pulpitis

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References
4. ZEHNDER, M., DELALEU, N., DU, Y., AND BICKEL, M., Cytokine, 22, no. 3-4, 2003, p. 84.

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