Interleukin-1 (IL1-α and IL1-β) in Gingival Fluid and Serum of Patients with Gingivitis and Periodontitis

SUMMARY

Objective. The purpose of the present study was to evaluate the levels of interleukin-1 (IL1-α and IL1-β) in gingival crevicular fluid (GCF) and serum, the important cytokines for initiation and progression of periodontal disease in healthy patients and patients with gingivitis and periodontal disease.

Material and Method. 90 individuals were subdivided into 3 groups of 30: control, gingivitis and periodontitis groups. GCF samples were obtained from 2 sites in each individual. Serum samples were also collected. Interleukin-1 (IL1-α, IL1-β) was evaluated using the commercial available ELISA technique.

Results. Mean gingival fluid levels of IL1-α and IL1-β in the 3 groups were, respectively: 19.39 pq/ml - 1.39 pq/ml in the control group; 28.27 pq/ml - 2.05 pq/ml in the gingivitis group; and 59.92 pq/ml - 5.25 pq/ml in the periodontitis group. Serum levels of IL1-α and IL1-β in the 3 groups were: 1.82 pq/ml - 0.09 pq/ml in the control group; 2.13 pq/ml - 0.35 pq/ml in the gingivitis group; and 2.46 pq/ml - 0.31 pq/ml in the periodontitis group. Levels of inflammatory cytokines in the gingivitis and periodontitis groups were significantly higher than in the control group (p< 0.05). In the serum, very low levels of cytokines were found. The level of serum IL1-α and IL1-β were, however, statistically significantly higher in the gingivitis and periodontitis groups (p<0.05).

Conclusion. Within the limits of this study, increased local production of immune inflammatory markers with increasing inflammation, and their monitoring in GCF, can help in the detection of the disease presence and/or its severity.

Keywords: Cytokines; Gingival Crevicular Fluid; Serum; Gingivitis; Periodontitis

Introduction

Cytokines have been defined as regulatory proteins produced by immune cells and other cells of the body. Their pleotropic action includes numerous effects on the cells of the immune system and modulation of inflammatory responses. The analysis of cytokine production levels has been also used as a tool for studying the local host response to a bacterial challenge.

Host response to periodontal pathogenic microorganism can be researched at many ways. Least invasive approach include analyzes of gingival crevicular fluid (GCF), inflammatory exudate which is released in the circumference of gingival sulcus. This exudate is a product of blood serum, and primary consists of inflammatory cells, much more noticeable polymorphonuclear leukocytes (PMN) and serums proteins.

Inflammatory cytokines are defined like cytokines (soluble proteins), which are induced as a result of inflammatory response and which are closely associated to the evolution and progression of the disease.

In particular, a large number of cytokines present in the GCF have been proposed as potentially useful diagnostic or prognostic markers of periodontal destruction. Among these, interleukin IL1-α and...
IL1-β have been shown to function in concert with other members of the cytokine network to regulate the cellular inflammatory response in the periodontium. This network comprises IL1-α, IL1-β, IL-6, IL-8, and TNF-α, which are generally classified as inflammatory cytokines and were observed to be of elevated levels in gingival fluid in patients with periodontal disease.

The aim of this study was to assess the relation between clinical parameters (such as dental plaque index and gingival inflammation index) and concentration of inflammatory cytokines (IL1-α, and IL1-β) within gingival crevicular fluid and serum samples with initiation and progression of periodontal disease in patients with gingivitis and periodontal disease.

**Material and Method**

At the Clinic of Oral Pathology and Periodontology, the University Dental Clinical Centre in Skopje, we examined 90 patients, divided in 3 groups. The first group, which represented the control group, consisted of 30 healthy patients without any sign of gingival or periodontal disease (verified clinically). Second group consisted of 30 patients with the diagnosed gingival disease, without signs of initial alveolar bone destruction (verified clinically and with radiogram). The third group consisted of 30 patients with the diagnosed initial periodontal disease (according to classification of AAP 1999, also verified clinically and with radiogram). The examined patients did not have any general disease and did not take antibiotic therapy in last 3 months.

All the examined patients undergone clinical and laboratories assays. Among clinical assays, we noted the dental plaque index (IDP - Silness-Loe) and gingival inflammation index (IGI - Loe-Silness). Laboratory assays for detection of gingival-fluid and serum levels of inflammatory cytokines (IL1-α and IL1-β) were realized at the Institute for Biology at the Faculty for Nature Sciences in Skopje. Gingival fluid was collected with filter perio-paper from mesiobucal surfaces of maxillary molars at the examined areas, with the action period of 30 seconds. Supragingival plaque was eliminated from teeth in the examined areas, and they were isolated to minimize possible salivary contamination. The gingival fluid was collected in micro-civettes with 0.5 ml phosphate buffered saline (pH=7.2) and then frozen on the -20°C till the day of the analysis. Before the analysis, the samples were centrifuged and then analyzed for IL1-α and IL1-β with commercially available ELISA method. Serum samples were also analyzed with Elisa method for IL1-α and IL1-β. The data were statistically evaluated with standard statistics parameters (computer programme "statistics for Windows"- 7).

**Results**

Results of the study are presented in tables and figures (graphs). Table 1 shows the age distribution in the examined groups. As it can be seen, healthy patients were younger than patients with gingivitis, while patients with periodontal disease were the oldest.

Clinical parameters are presented in table 2 (the IDP) and table 3 (the IGI). As it can be seen, both indexes confirmed the statistically significant differences between the healthy patients and patients with gingivitis and periodontal disease.

Figures 1 and 2 show mean values of gingival fluid levels of IL1-α and IL1-β in the examined groups, respectively. Analysis of variance (ANOVA) showed statistically significant differences in gingival fluid levels of both, IL1-α (F=56.50; p=0.00000) and IL1-β (F=36.029; p=0.00000), in the examined patients.

Figures 3 and 4 show mean values of serum levels of IL1-α and IL1-β in the examined groups, respectively. Analysis of variance (ANOVA) showed statistically significant differences in serum levels of IL1-α (F=232.89; p=0.00000) and IL1-β (F=109.259; p=0.00000) in the examined patients.

| Table 1. Age distribution of the examined groups (percentage values) |
|------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age of the examined groups | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 32 | 34 | 35 | 36 | total | % |
| Healthy | 5 | 10 | 5 | 10 | / | / | / | / | / | / | 30 | 17.6 |
| With gingivitis | / | / | 3 | 7 | 5 | 10 | 5 | / | / | / | 30 | 23.9 |
| With initial periodontal disease | / | / | / | / | / | / | 4 | 7 | 9 | 10 | 30 | 34.6 |
Table 2. The IDP values of the examined groups

<table>
<thead>
<tr>
<th>Examined groups</th>
<th>X</th>
<th>SD</th>
<th>df</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>0.33</td>
<td>0.47</td>
<td>29</td>
<td>3.80</td>
<td>0.000672*</td>
</tr>
<tr>
<td>With gingivitis</td>
<td>1.30</td>
<td>0.534</td>
<td>29</td>
<td>13.30</td>
<td>0.00000*</td>
</tr>
<tr>
<td>With initial periodontal disease</td>
<td>1.73</td>
<td>0.44</td>
<td>29</td>
<td>21.10</td>
<td>0.00000*</td>
</tr>
</tbody>
</table>

Table 3. The IGI levels of the examined groups

<table>
<thead>
<tr>
<th>Examined groups</th>
<th>X</th>
<th>SD</th>
<th>df</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>0.00</td>
<td>/</td>
<td>29</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>With gingivitis</td>
<td>1.233</td>
<td>0.43</td>
<td>29</td>
<td>15.70</td>
<td>0.00000*</td>
</tr>
<tr>
<td>With initial periodontal disease</td>
<td>2.93</td>
<td>0.25</td>
<td>29</td>
<td>63.32</td>
<td>0.00000*</td>
</tr>
</tbody>
</table>

Figure 1. Mean values of gingival fluid levels of IL1-α in the examined groups. ANOVA confirmed the statistically significant differences (F=56.50; p=0.00000)

Figure 2. Mean values of gingival fluid levels of IL1-β in the examined groups. ANOVA confirmed the statistically significant differences (F=36.029; p=0.00000)

Figure 3. Mean values of serum levels of IL1-α in the examined groups. ANOVA confirmed the statistically significant differences (F=232.89; p=0.00000)

Figure 4. Mean values of serum levels of IL1-β in the examined groups. ANOVA confirmed the statistically significant differences (F=109.259; p=0.00000)
Discussion

Gingivitis and periodontitis are defined as multifactor pathogen entities, which are initiated and assisted by bacterial colonization, but significantly modified by immune host response to bacterial plaque\(^{12}\).

When inflammatory response is generated in any tissue, the expression of different cytokines is usually increased, then starting deregulation of local immune response. Many research confirmed that non-restrictive production of cytokines leads to specific disease and eventual their progression. This also enhances the possibility of objective diagnosing the stage of inflammation through monitoring cytokine levels and their profile at the inflamed areas.

Gingivitis increases gradually in prevalence and severity from early childhood to the early teenage years, thereafter subsiding slightly and levelling off for the remainder of the second decade of life\(^{1,6,13}\). So far, only a few studies have considered age status as a modifying factor for variations in intracellular cytokine production that can be observed by comparing younger and aging adults, or children and adolescents\(^{3,10}\). Contradictory results have, however, been reported. Studies have shown both an increase and no change between different age groups examining alterations in proinflammatory systemic cytokine production. Healthy patients in our study had mean percentage age values of 17.6 years, patients with gingivitis 23.9 years, and patients with initial periodontal disease 34.6 years (Tab. 1).

The IDP of the examined groups showed statistically different levels in all the groups (Tab. 2). For the IGI (Tab. 3), we detected statistically significantly higher levels in the groups with gingivitis and initial periodontal disease (p<0.05) comparing to the healthy patients. Accordingly, differences in gingival fluid levels of IL1-\(\alpha\) were statistically significant among all the examined groups (Fig. 1). These levels were 19.3 pg/ml in healthy examinees, 28.27 pg/ml in examinees with gingivitis, and in examinees with initial periodontal disease levels they rapidly increased to 59.92 pg/ml. Levels of IL1-\(\beta\) in gingival fluid of healthy examinees were 1.39 pg/ml, and they increased in examinees with gingivitis to 2.05 pg/ml and in examinees with initial periodontal disease to 5.25 pg/ml, which was statistically significantly different (Fig. 2).

Serum concentration for these inflammatory cytokines (IL1-\(\alpha\) and IL1-\(\beta\)) were detected with very low levels. Our results are in accordance with Petrov at al\(^{13}\), who also confirmed continuously increased levels of IL1-\(\alpha\), and their relation to the increased plaque inflammation, pointing out the relevant role of IL1-\(\alpha\) and its presence in the gingival crevicular fluid as a sensitive marker for plaque induced gingival inflammation\(^{13}\). We also agree with findings of Orozo at al\(^{11}\), who indicated the increased local production of IL1-\(\beta\) in gingival fluid with the increased gingival inflammation. Similar results are reported by Preiss at al\(^{14}\) and Kinane at al\(^{8}\), who detected the increased levels of IL1-\(\alpha\) and IL1-\(\beta\) at the inflamed gingival tissue, and their extreme low concentrations in healthy individuals.

We suppose that this is the results of interaction between periodontal pathogenic microorganisms and the preserved host cells. This interaction activates the first step in the inflammatory response, cell activation in the connective tissue, and recruitment of neutrophil granulocytes, a stage that presents the initiation of early lesion in clinically evident gingival inflammation. First cells that are changed in this interaction are epithelial cells. They are really first cells which sustain changes from bacteria in gingival sulcus or periodontal pocket.

Bacterial adhesion activates secretion of pro-inflammatory mediators (IL1-\(\alpha\), IL1-\(\beta\), TNF-\(\alpha\)) from epithelial cells. In the same time, virulent factors that diffuse into the connective tissue, as well as inflammatory mediators produced by epithelial cells, stimulate the host cells in that area - monocytes/macrophages, fibroblasts and mast cells, to produce and release pro-inflammatory cytokines (IL1-\(\beta\), TNF-\(\alpha\), IL-6, IL-12), prostaglandin (PGE\(\_2\)), histamine and matrix-metalloproteinases (MMPs), which degrade the collagen from connective-tissue compartment. In the following clinical occurrence, the increased levels of IL1-\(\alpha\) and IL1-\(\beta\) continue the chain reaction of releasing many other inflammatory mediators, which furthermore recruit the inflammatory process. Confirmation of these activities is presented by histological verification of progressive lesion, which points out periodontal destruction.

Low serum concentration of inflammatory mediators in our study can be considered as a result of the fact that patients that were included in the study were with good health condition, so that their systemic influence of inflammatory mediators should not be expected.

Conclusion

The local production of interleukin (IL1-\(\alpha\) and IL1-\(\beta\)) in the gingival crevicular fluid increased with the increased gingival inflammation, which is the expression of the enhanced inflammatory response. This suggests that there is an association between the severity of plaque induced gingival inflammation and gingival fluid levels of these cytokines. So, we consider that they can be potent indicators of gingival and periodontal destruction.

References


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