Comparison of Gingival Crevicular Fluid Levels of IL-1β and IL-6 in Subjects with Gingivitis and Stage III Grade C Periodontitis

SUMMARY

Background/Aim: Periodontal diseases are inflammatory diseases that occur against microbial pathogens. Cytokines are biologically active molecules involved in this inflammatory process. This study aims to evaluate interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) cytokine levels in the gingival crevicular fluid (GCF) of individuals with stage III grade C (SIII-GC) periodontitis, gingivitis (G) and periodontally healthy (PH).

Material and Methods: A total of 64 individuals, including 22 PH, 22 G and 20 SIII-GC periodontitis were included in this study. Plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment loss (CAL) parameters were evaluated. GCF samples were analyzed by enzyme-linked immunosorbent assay (ELISA) kits.

Results: IL-1β and IL-6 levels in the GCF were significantly higher in the SIII-GC periodontitis group compared to the other groups (P <0.05). There was no significant difference between IL-1β and IL-6 levels in the PH and G groups (P > 0.05). GCF IL-1β and IL-6 levels were positively associated with the whole mouth and sampling area clinical periodontal parameters (P < 0.001).

Conclusions: GCF IL-1β and IL-6 total amounts are effective in determining the regions and individuals under risk in SIII-GC periodontitis. Moreover, GCF IL-1β and IL-6 levels were seen to be effective determinants in differentiating gingivitis and periodontitis.

Key words: Gingival Crevicular Fluid, Interleukins, Interleukin-6, Macrophages, Periodontitis

Introduction

Periodontitis is a chronic disorder, that influences the supporting periodontal tissues. Although the presence of pathogens is necessary for developing periodontitis, it is insufficient by itself and the host response is critical for disease initiation. As a result of the host response to periodontal pathogens, various inflammatory cytokines are released from periodontal tissues.

Interleukin-1 beta (IL-1β) is a multifunctional pleiotropic cytokine produced by monocytes, macrophages, natural killers, and B cells. It has been reported that IL-1β increases blood flow with leukocyte accumulation and neutrophil infiltration at the inflammation site. Studies demonstrated that IL-1β raises the release of collagenolytic enzymes and matrix metalloproteinases, that causes matrix deterioration and leads to bone and tissue destruction. Furthermore, it has been reported that IL-1β affects the RANK/RANKL/OPG system associated with bone metabolism. In addition to these direct effects, IL-1β causes the increase of pro-inflammatory cytokine levels such as IL-6 and IL-8, which cause bone destruction from fibroblasts. IL-1β level increases in periodontitis. Compared to healthy patients, high levels of IL-1β were found in the gingival crevicular fluid (GCF) and saliva taken from periodontitis patients.

IL-6 is a cytokine with many functions that are released from various cells, such as monocytes, macrophages, fibroblasts which affect the immune response.
response and inflammatory reactions that occur against the antigen. It is also the leading inducer of factors, such as C-reactive protein, fibrinogen and serum amyloid a protein, and plays an important role in inflammation. In hematopoiesis, IL-6 similarly perform with IL-3 and stimulates macrophage and megakaryocyte differentiation. Furthermore, IL-6 has been reported to exhibit impaired leukocyte accumulation at the inflammation site, causing imperfect transition into a chronic mononuclear cell infiltration. Tumor necrosis factor-α and IL-1β activate the transcription factor for IL-6 production. Studies reported that IL-6 release is involved with the severity of periodontal disease and high amounts of IL-6 are present in GCF taken from gingivitis (G) and periodontitis patients. IL-1β and IL-6 levels in GCF were investigated in individuals with periodontitis in previous studies according to the old classification; however, there are no studies in which these biomarkers were investigated according to the new classification criteria. The aim of our study is to evaluate the IL-1β and IL-6 levels in GCF in individuals with stage III and grade C (SIII-GC) periodontitis and G for the new classification criteria and to compare them with periodontally healthy (PH) individuals.

Material and Methods

Research group and clinical evaluation

Between December 2020 and March 2021, 64 volunteers aged between 28 and 48 (mean age = 36.83 ± 4.32) were included in the study. This study was conducted as per the Declaration of Helsinki and was adopted by the Ethics Committee of Usak University Faculty of Medicine (117-09-09). It was recorded in the clinical studies registry (NCT04689438). Written consent was obtained from all individuals by explaining the purpose and design of the study.

After taking anamnesis from the patients, oral examination was performed. Individuals with a systemic disease such as diabetes and immunological diseases that would affect the inflammatory state, those who smoked, were pregnant or breastfeeding, taking medication that would affect the periodontium in the last six months, and those who were receiving a periodontal therapy were excluded from the study. Volunteer individuals who did not have any systemic disease, did not smoke, and had at least 16 permanent teeth in their mouth were incorporated in the study.

All periodontal assessments were conducted with a manual probe (Williams, Hu-Friedy, Chicago, IL) by an experienced calibrated periodontist (AD). Plaque index (PI), gingival index (GI) and presence of bleeding on probing (BOP) were measured at four sites. Periodontal probing depth (PPD), and clinical attachment loss (CAL) were measured at six points per tooth apart from the third molar.

Calibration exercise was performed on five patients with periodontitis who were not included in the study, and the intra-examiner reliability tested with kappa coefficient was 0.90 for PPD and 0.88 for CAL.

After clinical evaluation, the persons were separated into three groups according to the new classification published in 2017:

1. PH control group (n = 22): It comprises individuals with clinically healthy gingiva on a stable periodontium with a BOP of <10%, a PPD ≤ 3 mm, and no attachment or bone loss.

2. Group G (n = 22): It comprises individuals with a BOP of 10% and above, a PPD ≤ 3 mm and no attachment or bone loss (plaque induced gingivitis).

3. SIII-GC periodontitis group (generalized) (n = 20): Individuals with an interdental CAL ≥ 5 mm, PPD ≥ 6 mm with radiographic bone destruction continuing to the middle or apical part of the root and a maximum loss of 4 teeth because of periodontitis were considered as group of stage III periodontitis (in 30% and more regions). During the diagnosis of periodontitis, care was taken that CAL did not originate from gingival recession of traumatic origin, tooth decay extending to the cervical of the tooth, CAL seen in the distal of the second molar associated with the extraction or malposition of the third molar, lesion in the marginal periodontium of endodontic origin, or vertical root fracture. Radiographic bone destruction was assessed from the tooth, which demonstrated the very serious bone destruction as a proportion of root length. If there is rapid bone loss compared to the biofilm and the % of root bone loss /age > 1.0; it was determined as grade C16,17,18.

GCF sampling procedure

GCF samples were obtained in the morning hours 24-48 h after the clinical periodontal evaluation. GCF was obtained from the buccal part of the two-interdental area of single rooted teeth in each jaw using paper strips (Periopaper; Proflow). In periodontal healthy control group, GCP was obtained from the regions without BOP (plaque induced gingivitis)17.

Individuals with an interdental CAL ≥ 5 mm, PPD ≥ 6 mm with radiographic bone destruction continuing to the middle or apical part of the root and a maximum loss of 4 teeth because of periodontitis were considered as group of stage III periodontitis (in 30% and more regions). During the diagnosis of periodontitis, care was taken that CAL did not originate from gingival recession of traumatic origin, tooth decay extending to the cervical of the tooth, CAL seen in the distal of the second molar associated with the extraction or malposition of the third molar, lesion in the marginal periodontium of endodontic origin, or vertical root fracture. Radiographic bone destruction was assessed from the tooth, which demonstrated the very serious bone destruction as a proportion of root length. If there is rapid bone loss compared to the biofilm and the % of root bone loss /age > 1.0; it was determined as grade C16,17,18.
**Determination of IL-1β and IL-6 levels in GCF**

IL-1β and IL-6 levels in GCF were determined as per the manufacturer’s instructions for enzyme-linked immunosorbent assay (ELISA) kits (Human IL-1β ELISA kit and Human IL-6 ELISA kit-Elabscience, Texas, USA). Before GCF specimens were inserted into holes coated with antibodies specific for IL-1β and IL-6, the standards in the kits were diluted for the manufacturer’s instructions. A stop solution was inserted to every hole and the absorbance amounts were defined by a spectrophotometric ELISA reader (Microplate Reader; Biotek, Winooski). Total levels of IL-1β (pg/30s) and IL-6 (pg/30s) collected in 30 s were determined. The lowest detection limits were reported to be 2.69 and 1.06 ng/mL for IL-1-and IL-6.

**Statistical analysis**

In the study, G Power 3.1 package software was utilized to assess the adequate specimen volume. As per calculations, the sample size that would provide a Type I error of 0.05 was determined as at least 20 persons for each group with an effect size of 0.90 and test power of 80% in a one-way ANOVA study. To assess the statistical method to be utilized, the Kolmogorov–Smirnov and Shapiro–Wilk tests were made to test whether the variables follow a normal distribution. A critical value of p=0.05 was used. According to the data obtained, if the p value was <0.05, the data showed normal distribution; if it was >0.05, the data showed normal distribution. In comparison between the groups, Kruskal–Wallis test was used as a nonparametric method because the data did not show normal distribution. The frequency data were evaluated by the chi-square test. The association between clinical parameters and IL-1β and IL-6 levels in GCF was evaluated by the Spearman’s rank correlation analysis. The data were evaluated by the statistical software program SPSS (v. 22.0, IBM).

**Results**

Table 1. shows the distribution of demographic data and clinical parameters (the whole mouth and sampling site) of 64 individuals included in the study. Moreover, 59.4% of participants are female and 40.6% are male; there was no significant distinction among the participants in the PH, G and SIII-GC periodontitis groups (P > 0.05).

While the whole mouth and sampling site GI and BOP parameters were significantly higher in the SIII-GC periodontitis and G groups compared to the PH control group (P < 0.05), these parameters were reported similar in the SIII-GC periodontitis and G groups. PI, PPD and AL parameters were reported to be significantly higher in the SIII-GC periodontitis group compared to the G and PH control group (P < 0.05) (Table 1).

**Table 1. The demographic characteristics and clinical periodontal parameters of the study groups**

<table>
<thead>
<tr>
<th>Demographic variables</th>
<th>PH (n=22)</th>
<th>G (n=22)</th>
<th>SIII-GC (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.5±5.21</td>
<td>36.3±5.40</td>
<td>37.7±4.23</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>17/5</td>
<td>11/11</td>
<td>10/10</td>
</tr>
<tr>
<td><strong>Periodontal parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole mouth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD (mm)</td>
<td>1.88±0.16</td>
<td>2.18±0.25</td>
<td>6.48±0.28*</td>
</tr>
<tr>
<td>AL (mm)</td>
<td>0</td>
<td>0</td>
<td>5.18±0.27*</td>
</tr>
<tr>
<td>GI</td>
<td>0.15±0.10</td>
<td>1.95±0.24</td>
<td>2.04±0.19*</td>
</tr>
<tr>
<td>BOP (%)</td>
<td>1.79±0.22</td>
<td>74.92±5.84</td>
<td>77.08±3.47*</td>
</tr>
<tr>
<td>PI</td>
<td>0.32±0.22</td>
<td>1.60±0.33</td>
<td>2.88±0.21*</td>
</tr>
<tr>
<td><strong>Sampling site</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD (mm)</td>
<td>1.99±0.17</td>
<td>3.00±0.10</td>
<td>6.79±0.50*</td>
</tr>
<tr>
<td>AL (mm)</td>
<td>0</td>
<td>0</td>
<td>5.32±0.32*</td>
</tr>
<tr>
<td>GI</td>
<td>0</td>
<td>0</td>
<td>2.25±0.12*</td>
</tr>
<tr>
<td>BOP (%)</td>
<td>0</td>
<td>100</td>
<td>100*</td>
</tr>
<tr>
<td>PI</td>
<td>0.26±0.18</td>
<td>2.05±0.50</td>
<td>2.96±0.14*</td>
</tr>
</tbody>
</table>

Abbreviations: PH, periodontally healthy; G, gingivitis; stage III grade C periodontitis, SIII-GC; PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; AL, attachment loss. All data (except gender) are given as mean ± SD.

*Significantly different from PH
‡Significantly different from G
*Significantly different from SIII-GC periodontitis

Figure 1. shows the mean difference in total IL-1β and IL-6 levels in GCF. While total IL-1β and IL-6 levels in GCF were reported to be significantly higher in the SIII-GC periodontitis group compared to the G and PH control groups (P<0.05), total IL-1β and IL-6 levels were reported to be similar in the PH control group and the group G (P > 0.05).

**Table 2.** shows the relationship between the total IL-1β and IL-6 levels and the clinical parameters in the whole mouth and sampling site. GCF IL-1β and IL-6 total
levels were positively associated with the whole mouth and sampling area clinical periodontal parameters (PI, GI, BOP, PPD and CAL) (P < 0.001).

Table 2. Correlations between total GCF IL-1β and IL-6 levels with clinical parameters of study groups

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>IL-1β (pg/30s)</th>
<th>IL-6 (pg/30s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole mouth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>0.461**</td>
<td>0.537**</td>
</tr>
<tr>
<td>GI</td>
<td>0.340**</td>
<td>0.249**</td>
</tr>
<tr>
<td>BOP</td>
<td>0.336**</td>
<td>0.273**</td>
</tr>
<tr>
<td>PD</td>
<td>0.610**</td>
<td>0.550**</td>
</tr>
<tr>
<td>CAL</td>
<td>0.568**</td>
<td>0.518**</td>
</tr>
<tr>
<td>Sampling site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>0.490**</td>
<td>0.400**</td>
</tr>
<tr>
<td>GI</td>
<td>0.348**</td>
<td>0.294**</td>
</tr>
<tr>
<td>BOP</td>
<td>0.323**</td>
<td>0.283**</td>
</tr>
<tr>
<td>PD</td>
<td>0.570**</td>
<td>0.548**</td>
</tr>
<tr>
<td>CAL</td>
<td>0.573**</td>
<td>0.498**</td>
</tr>
</tbody>
</table>

Abbreviations: IL-1β, interleukin-1 beta; IL-6, interleukin-6; PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; AL, attachment loss. Spearman’s rank correlation test. ** p<0.001. All data are given as correlation coefficient.

**Discussion**

Periodontal diseases were reclassified as periodontal and peri-implant diseases and conditions at the World Workshop in 2017. With this classification, a new perspective was introduced to periodontal diseases, periodontal health was redefined and periodontitis was reclassified according to its severity and rate of progression. This study focuses on the change in IL-1β and IL-6 levels within the framework of the new classification; it was thought that they could be a biomarker that can be included in the new classification of periodontal diseases and conditions. This study aims to assess the alterations GCF IL-1β and IL-6 levels in PH, G and SIII-GC periodontitis patients as per the new classification. To our information, this is the first report to analyze the change in IL-1β and IL-6 levels in GCF according to the new classification criteria.

In this study, total IL-1β level in GCF was reported significantly higher in the SIII-GC periodontitis patients compared to the G and PH control groups. In studies conducted by Becerik et al. and Toyman et al., it was reported that periodontitis patients have higher IL-1β amounts in their GCF compared to healthy patients. It was also reported that periodontitis patients with advanced PPD and AL in our study shows IL-1β activity in regions with deep periodontal pockets in periodontal disease. Similar to previous studies, the high amounts of IL-1β in the SIII-GC periodontitis patients with advanced PPD and AL in our study shows IL-1β activity in regions with deep periodontal pockets in periodontal disease. The increased degree of IL-1β in GCF indicates the function of IL-1β in the inflammatory reaction that occurs in the development of periodontal disease, which leads to CAL and increased bone loss.

Takahashi et al. that IL-6 is reported in endothelial cells, fibroblasts and macrophages of periodontitis patients and not in individuals with healthy gingiva. In clinical studies, IL-6 acts a crucial function in the inflammatory reaction to gram-negative bacteria, which affects the subgingival microbial formation and susceptibility increases to pathogenic bacteria. It was reported by Hughes et al. that IL-6 is a very important stimulant of bone resorption and osteoclast differentiation in periodontitis. It was reported by Moreira et al. that IL-6 release is positively associated with CAL, and responsible for ongoing tissue destruction in periodontitis. In another study, it was shown that IL-6 polymorphism alters the immune response and increases susceptibility to aggressive periodontitis where additional destruction occurs. In the study conducted by Reis et al., it was reported that IL-1α, IL-1β, and IL-6 cytokines in GCF were reported at high levels in periodontitis patients.

In our study, IL-6 level in GCF was reported to be significantly higher in the patients with SIII-GC periodontitis who had a high disease progression rate, advanced attachment and bone loss, compared to the PH and G patients. This is considered to be attributed to IL-6 playing a function in the pathogenesis of periodontitis by getting advanced attachment and bone loss in individuals with advanced stage and grade degrees.

In this study, BOP, which is a very important inflammation marker in the new classification, were reported to be significantly higher, along with GI, in the periodontitis and G groups compared to the PH group; it was found similar in the periodontitis and G groups. This is thought to be due to the GI and BOP showing only clinical level of inflammation.

In this study, the clinical parameters of PI, PPD and CAL were reported to be significantly higher in the periodontitis patients compared to the PH and G patients. This is thought to be the result of attachment and bone loss with further progression of inflammation, which begins with increased plaque accumulation.

In our study, a positive correlation was reported between clinical periodontal parameters and total IL-1β and IL-6 levels. Clinical parameters increase in periodontitis in accordance with IL-1β and IL-6. The data obtained show that there is a positive correlation between IL-1β and IL-6 levels and inflammation. Moreover, it is thought that increased IL-1β and IL-6 levels in periodontitis are indicative of tissue response to inflammation.

The enzyme level in the gingival crevicular fluid is determined as total and concentration. In previous studies, it was showed that high GCF amount in diseased areas will decline the concentration of the GCF component.
Moreover, it was shown that it would be more appropriate to present the data in total to assess the connection between GCF components and periodontal diseases\textsuperscript{39}. In our study, GCF IL-1β and IL-6 levels were expressed as total because calculating the total amount of markers would be a good, acceptable and credible marker for diagnostic aims.

Different biological fluids such as GCF, blood, and saliva are used to determine potential markers in periodontal diseases\textsuperscript{30}. GCF is a region-specific fluid containing host factors\textsuperscript{31}. In our study, GCF was used to assess the marker level in periodontal disease. According to our results, GCF is a fluid capable of showing IL-1β and IL-6 levels in SIII-GC periodontitis.

This study has certain limitations. Firstly, although it is sufficient according to power analysis, the number of individuals included in the study is small. Moreover, serum and saliva samples were not taken from individuals participating in the study. Finally, periodontitis groups at different stages were not incorporated in this work.

Conclusions

IL-1β and IL-6 levels in GCF are considered to have great potential in detecting SIII-GC periodontitis. Increased IL-1β and IL-6 levels were reported to be associated with SIII-GC periodontitis and clinical parameters. Moreover, these markers were reported to differentiate between gingivitis and periodontitis. Based on the consequences received in this study, in defining question-marked biological markers in the new classification, IL-1β and IL-6 levels in GCF can be used as promising markers to identify regions and individuals at risk in SIII-GC periodontitis.

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References


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Correspondence
Ahu Dikilitaş
Department of Periodontology
Faculty of Dentistry, Usak, Turkey
e-mail: ahu.dikilitas@usak.edu.tr