Serum Cytokines Profiles and Some Salivary Parameters in Chronic Periodontitis Patients in Mosul - Iraq

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Abstract

The aim of study is to measure serum IL-1 β, IL-6, IL-8, IFN-γ and TNF-α levels, and some of salivary parameters like sIgA, total protein and peroxidase specific activity in patients with chronic periodontitis in Mosul and compared them with control group. The study population consisted of 91 patients suffering from chronic periodontitis, aging between ≤18-73 years old and 18 control samples collected from healthy individuals ranged between 23-35 years old. Blood and saliva samples were collected from patients and control groups to measure serum IL-1β, IL-6, IL-8, IFN-γ and TNF-α, and salivary sIgA, total protein levels and peroxidase specific activity. In the present study most of patients were showed localized chronic periodontitis, ranging from severe 57% to moderate infection 43%. Salivary sIgA level showed significant increase in chronic periodontitis comparing with control group. Patients group showed significance correlation between serum TNF-α with serum IL-1β, IL-6 and IFN-γ levels. Also serum IL-1β level showed significant correlation with salivary sIgA and total protein levels. Furthermore negative correlations were detected between salivary sIgA and serum IL-8, as well as between salivary total protein and peroxides specific activity.

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According to periodontal pocket depth, serum IL-1β, IFN-γ, salivary sIgA levels and salivary peroxidase
specific activity showed significance increase in patients with 7mm pocket depth. In the present study, most of patients showed localized chronic periodontitis. Salivary sIgA level showed significant increase in chronic periodontitis comparing with control group. Serum IL-1β, IFN-γ, salivary sIgA levels and salivary peroxidase specific activity were linked with 7mm periodontal pocket depth.

**Keywords:** Periodontitis, pocket depth, cytokines, and sIgA.

1. **Introduction**

Chronic periodontitis is an infectious disease caused by specific pathogens that resist the host defenses, and chemotherapy. These pathogens are pioneer organisms that colonize the region of cervical gingiva and alter the periodontal environment to facilitate colonization of secondary organisms, which are usually more virulence than pioneer one [1]. The initial host immune system response to infections depends on local inflammatory reaction which activates innate immune system [2]. At the cellular level, the recognition and reaction with infectious agents include macrophages, dendritic cells and natural killer cells, neutrophils, osteoclasts and furthermore humeral response via T-Cells and B-cells [3,4].

Traditionally bacterial infection has an essential role in periodontal infection [5], but periodontum tissues destruction as a result of host immune system response to presence of pathogens via pro-inflammatory cytokines production within periodontal tissue and periodontal sulcus fluid, including interleukin-1beta (IL-1β), interleukin-6 (IL-6), matrix metallo proteases (MMPs), prostaglandin E2 (PGE2), tumor necrosis factor-alpha (TNF-α), and immunoglobulins [6,7]. The elevation of inflammatory mediators with failure to encapsulate this inflammatory within gingival tissue leads to expansion of the response adjacent to underlying tissues and destroy the supporting periodontal tissues [8].

2. **Materials and Methods**

2.1. **Subject Groups**

The subjects in this study were 91 patients with chronic periodontitis aging between ≥18 - 73 years old, (57 males aged 18 to 73 years, mean age, 37.33 years, and 34 females aged 18 to 63 years, mean age, 32.14 years. Patients referred to Periodontal Clinic in the College of Dentistry/Mosul University from January 2011 to April 2012.

2.2. **Control Group**

This group consisted of 18 clinically healthy ranged between 23-35 years old. (18 males aged 23 to 35 years and 3 females aged 23 to 30 years) working in Mosul University. This group had no signs of any systemic disease, gingivitis or any type of periodontitis. All patients and the healthy individuals underwent an oral examination and selected by a specialist. Patients with chronic periodontitis status have >3-≥7mm pockets depth, according to the classification of the periodontal diseases issued by the American Academy of Periodontology in 1999 [9].
2.3. Data Source

Case sheet was performed especially for the purpose of examination.

2.4. Sample Collecting

Periodontitis patients had no clinical evidence of previous periodontal destruction with more than 3 mm probing depth at 3 or more sites, while the healthy controls had no clinical evidence of periodontal disease, healthy gingival sites showed neither periodontal attachment loss nor gingival inflammation and exhibited periodontal probing depth equal to or less than 3 mm.

2.4.1. Serum Collecting

5ml of blood was collected from patients and control groups, serum was dispensed by centrifugation at 2500 rpm for 10 min and aliquoted in a sterile plastic Eppendorff tubes (0.5ml) and stored at -20°C until the time of analysis [10].

2.4.2. Collection of Saliva

Unstimulated saliva was collected from patients and control groups via passive drooling into a sterilized disposable collector cup. Saliva was centrifuged at 3000 rpm for 10 min. The clear supernatant fraction was then separated and dispensed in Eppendorff tubes and stored at -20°C until required for analysis [11].

2.5. Materials and Kits Used in Serological Tests

1. Human Interleukin-1β ELISA kit, ABO Switzerland Co., Ltd. (China).
2. Human Interleukin-6 ELISA kit, ABO Switzerland Co., Ltd. (China).
3. Human Interleukin-8 ELISA kit, Immune Leader, (China).
4. Human interferon-γ ELISA kit, ABO Switzerland Co., Ltd. (China).
5. Human Tumor Necrosis Factor-α ELISA kit, ABO Switzerland Co., Ltd. (China).

2.6. Materials and Kit Used for Salivary Serological and Biochemical Study

1. Salivary sIgA ELISA kit, Dia Metra, (Italy).
2. Saliva total protein biochemical test kit, BIOLABO S.A., (France).
2.7. Statistical Analysis

T-Test, Pearson Chi-Square Test, Pos Hoc Test, ANOVA Test, Duncan Test, Correlations, Mean, Std. Deviation, Std. Error.

3. Results

In the present study most of patients showed localized chronic periodontitis, 57% of them suffered from severe infection (≥5 mm pocket depth), and 43% of them have moderate infection (≥3-4 mm pocket depth). (Figure 1)

![Figure (1): Infection status according to pocket depth](image)

3.1. Comparison between chronic periodontitis and control groups

The comparison between chronic periodontitis and control groups using serum IL-1β, IL-6, IL-8, IFN-γ and TNF-α levels and some of salivary parameters like sIgA, total protein and peroxidase specific activity showed increase in the above parameter levels, but statistically not significant, except salivary sIgA levels which was statistically significant (Table 1).
Table (1): Comparison of some cytokines and salivary parameters between chronic periodontitis and control groups

<table>
<thead>
<tr>
<th></th>
<th>Control n(18)</th>
<th>Patients n(91)</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± Std. Deviation</td>
<td>Mean ± Std. Deviation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL- 8 pg/ml</td>
<td>25.055 ± 6.109</td>
<td>59.195 ± 26.560</td>
<td>0.514</td>
<td>Non</td>
</tr>
<tr>
<td>IL- 6 ng/ml</td>
<td>13.366 ± 1.851</td>
<td>21.810 ± 18.041</td>
<td>0.260</td>
<td>Non</td>
</tr>
<tr>
<td>IL- 1β pg/ml</td>
<td>26.666 ± 4.8442</td>
<td>76.0736 ± 41.864</td>
<td>0.629</td>
<td>Non</td>
</tr>
<tr>
<td>IFN-γ ng/L</td>
<td>300.666 ± 16.415</td>
<td>453.948 ± 397.130</td>
<td>0.607</td>
<td>Non</td>
</tr>
<tr>
<td>TNF-α ng/L</td>
<td>280.833 ± 20.143</td>
<td>333.1263 ± 279.301</td>
<td>0.651</td>
<td>Non</td>
</tr>
<tr>
<td>Salivary sIgA µg/ml</td>
<td>42.549 ± 14.0799</td>
<td>69.266 ± 27.0799</td>
<td>0.019</td>
<td>Significance</td>
</tr>
<tr>
<td>Salivary total protein mg/ml</td>
<td>2.701 ± 0.860</td>
<td>3.2937 ± 1.6351</td>
<td>0.383</td>
<td>Non</td>
</tr>
<tr>
<td>Salivary peroxides specific activity u/mg</td>
<td>0.01530 ± 0.0091</td>
<td>0.05411± 0.05243</td>
<td>0.098</td>
<td>Non</td>
</tr>
</tbody>
</table>

3.2. Correlation between some of cytokines and parameters within periodontitis patients group

The present study showed significant association among cytokines and salivary parameters within patients group as summarized below:
First, presence of significance correlation between serum TNF-α with serum IL-1β, IL-6 and IFN-γ levels. Second, serum IL-1β level showed significant correlation with salivary sIgA and total protein levels. Finally negative correlation detected between salivary total protein and peroxides specific activity, and between salivary sIgA with IL-8 (Tables 2).

Table (2): Correlation between some of cytokines and parameters within periodontitis group.

<table>
<thead>
<tr>
<th></th>
<th>IL-8</th>
<th>IL-6</th>
<th>IL-1β</th>
<th>IFN-γ</th>
<th>TNF-α</th>
<th>Saliva sIgA</th>
<th>Saliva total protein</th>
<th>Saliva peroxides Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-8</strong></td>
<td>1</td>
<td>0.206</td>
<td>0.052</td>
<td>0.122</td>
<td>0.768**</td>
<td>-0.359**</td>
<td>0.076</td>
<td>0.069</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>1</td>
<td>0.331*</td>
<td>0.312*</td>
<td>0.457**</td>
<td>0.117</td>
<td>0.0202</td>
<td>-0.033</td>
<td></td>
</tr>
<tr>
<td><strong>IL-1β</strong></td>
<td>1</td>
<td>0.711**</td>
<td>0.398**</td>
<td>0.276*</td>
<td>0.478**</td>
<td></td>
<td></td>
<td>-0.089</td>
</tr>
<tr>
<td><strong>IFN-γ</strong></td>
<td>1</td>
<td>0.474**</td>
<td>0.260</td>
<td>0.491**</td>
<td></td>
<td></td>
<td></td>
<td>-0.134</td>
</tr>
<tr>
<td><strong>TNF-α</strong></td>
<td>1</td>
<td></td>
<td>-0.102</td>
<td>0.256</td>
<td></td>
<td></td>
<td></td>
<td>-0.016</td>
</tr>
<tr>
<td>Saliva sIgA</td>
<td>1</td>
<td>0.264*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.141</td>
</tr>
<tr>
<td>Saliva total protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary peroxides Specific activity</td>
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</tbody>
</table>

**. Correlation is significant at the 0.01 level. *. Correlation is significant at the 0.05 level. n.= 91

3.3. Relationships between parameter concentrations and periodontal pocket depth in periodontitis patients group

The comparison between some parameter levels and their relation with periodontal pocket depth within periodontitis group, showed no statistical significance differences between serum IL-6, IL-8, TNF-α levels and salivary total proteins concentration. (Figures 2). However IL-1β and IFN-γ levels showed statistically significant increase in patients with periodontal pocket depth 7mm, whereas salivary sIgA showed significant increase in patients with both 7mm and 3mm pocket depth (Figure 3). The relationship between salivary peroxidase specific activity and periodontal pocket depth showed significant increase in patients with 7mm pocket depth. However there was no significance variation in its activity with other depths (Figure 3).
Figure (2): Relationships between serum parameters concentrations and periodontal pocket depth in periodontitis patients

Figure (3): Relationships between salivary parameters concentrations and periodontal pocket depth in periodontitis patients group
5. Discussion and conclusions

In this study most of patients showed localized chronic periodontitis, 57% of them suffered from severe infection and 43% of them have moderate infection. In chronic periodontitis the periodontal epithelium surface exposed to invasion pathogens ranged between 8-20 cm², therefore chronic and aggressive periodontitis are similar to systemic infection [12].

The Comparison between chronic periodontitis and control groups showed no significant differences in serum IL-1β, IL-6, IL-8, IFN-γ, TNF-α levels, total protein concentration and peroxidase specific activity. Periodontitis inflammatory reaction is initiated as a response to the presence of pathogens and their products which stimulate a cellular immune system to release cytokines and other mediators leading to extinction of the inflammation throughout the gingival tissues reaching the connective tissue and alveolar bone [13]. Thus the inflammatory bone resorption depends upon the concentration of inflammatory mediators that reaching the alveolar bone [2]. IL-1β is a pro-inflammatory cytokine which regulates IL-6 production and stimulates bone resorption [14,15]. These foundations support the observations of the current study. IL-6 plays a regulatory key role in chronic periodontitis via modulating periodontal local host responses [16]. Raunio and his colleagues observed a significant increased in serum IL-6 level in chronic periodontitis patients comparing with control groups [17], but in the present study there was no significant differences, such differences may depend on several factors: First, the severity of periodontits in the present study, most of patients showed localized chronic periodontitis, 57% of them suffered from severe infection (≥5 mm pocket depth), and 43% of them have moderate infection (≥3-4 mm pocket depth). Second, the subjects in the control group may have subclinical diseases which confused the result. Generally IL-6 level could be used as biomarker to determine gingivitis and periodontitis susceptibility and periodontal therapy prognosis [18].

IL-8 is a chemokine increases as a result of any inflammatory conditions which chemoattract and increase neutrophils phagocytic activity against invading agents [19, 20]. The current result showed agreement with Ladez and his colleagues who observed no significant difference in serum IL8 level between periodontitis and healthy groups [21].

Andrukhov and his colleagues observed the presence of positive association between serum TNF-α level and periodontal inflammation [22]. TNF-α is an early cytokine which induces production of IL-1β, IL-6 and enhances migration of inflammatory cells to the site of inflammation [23]. Also TNF-α stimulates production of matrix metallo proteinases, which enhances bone loss [24, 25]. Thus non significance variations in the present result may be associated with infection status. TNF-α is a pro-inflammatory cytokine which is increased in the early infection status and deceased with time as a result of IL-6 activity which acts as an anti-inflammatory cytokine [26].

IFN-γ has immune modulator properties, which is produced as a part of the innate immune response against infections [27]. Kobayashi and his colleagues didn’t observe a significance association between serum TNF-α
level and periodontal infection, but it is linked with IL-12 level [28]. This concept may support the current results.

Salivary sIgA level shows significance increase in its level within periodontitis patients comparing with control groups. Similar observation has been achieved by others [29]. Salivary sIgA is considered as one of the main immune defense mechanism in saliva via binding to soluble and particulate antigens [30] as well as it inhibits various enzymes and bacterial colonization on oral hard surfaces [30]. Therefore Olayanju and his colleagues suggested the use of salivary sIgA level elevation as a screening tool for periodontitis [31].

Salivary peroxidase specific activity showed increase in periodontitis patients than control group. Peroxidase enzyme protects oral cavity via inhibiting bacterial growth and preventing hydrogen peroxide and dental biofilm acid accumulation [32]. Although its increase is not significance but this result is similar to Guven and his colleagues observation who noted that salivary peroxidase remained constant within a normal range in several diseases [33], but its elevation in periodontitis patients may be an indication of periodontal disorder [34].

In this study the correlation between some of cytokines and other parameters in periodontitis patients were showed a significance correlations between serum TNF-α with serum IL-1β, IL-6 and IFN-γ levels. Several studies demonstrated that IL-1β, IL-6, IFN-γ and TNF-α, induce several reactions associated with inflammation, tissue destruction and bone resorption [18,35,36]. Periodontitis like other inflammatory disease can also affect on cytokines concentrations, thus periodontal infections depend on fluctuation of mediators levels, expression of pro-inflammatory cytokines, which play a critical role in the destruction of connective tissues and alveolar bone that support the teeth [37]. But expression of anti-inflammatory cytokines and other mediators serve as bone resorption inhibitor, such as IFN-β and IFN-γ [38]. In the present study the comparison between some parameter levels and their relation with periodontal pocket depth showed no statistical significance between serum IL-6, IL-8, TNF-α levels and salivary total proteins concentration, within periodontitis group. IL-1β, IFN-γ, salivary sIgA, levels and salivary peroxidase specific activity showed statistically significant increase in patients with periodontal pocket depth 7mm. However there were no significance variations in its activity with other depths. Górska and his colleagues observed clear relationship between clinical parameters and IL-1β, TNF-α and IFN-γ concentrations within inflamed gingival tissues and serum samples of severe chronic periodontitis patients and suggested that they have an important role in the initiation and progression of periodontal disease [39].

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