SOME IMMUNOLOGICAL ASPECTS OF IRAQI PATIENTS WITH PERIODONTAL DISEASES

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ABSTRACT

Background: Periodontal disease could be defined as a disorder of supporting structures of teeth, including the gingiva, periodontal ligament and alveolar bone. Periodontal disease develops from a pre-existing gingivitis. Systemic alterations of the cellular and humoral immune responses to periodontal pathogens among chronic periodontitis patients have been evaluated, including immunosuppression, exaggerated inflammatory cell responses, impaired neutrophils, and antibody production. Methodology: One hundred eighteen samples were examined in the present study. Complements C3 and C4 and immunoglobulins IgA, IgM and IgG proteins were determined by radial immunodiffusion (RID) plate containing a specific antibody will form an immuno-complex, visible as a ring around the well. IgE was determined by ST AIA PACK IgE II kit (manufactured by Tosoh, Japan). Interleukins in periodontal patient were determined by quantikine ELISA kit (Human IL-4 and Human IL-10; Manufactured by R&D system, USA). Results: It was found significant positive linear correlation between C3 and C4, C4 and IgA, and significant negative correlation between C4 and IL-10 (p<0.05). Conclusions: The immunological analysis results showed that the comparison of serum IgA, IgM and IgG; C3 and C4; IL-4 and IL-10 concentrations between patients and control groups were significant (p<0.05). It was found significant positive linear correlation between C3 and C4, C4 and IgA, and significant negative correlation between C4 and IL-10 (p<0.05).

KEYWORDS: Periodentitis, cytokines, immunoglobulins, complements.
1. INTRODUCTION

Periodontal disease could be defined as a disorder of supporting structures of teeth, including the gingiva, periodontal ligament and alveolar bone. Periodontal disease develops from a pre-existing gingivitis. However, not every case of gingivitis develops into a periodontal disease. The inflammation of gingiva alone is termed gingivitis, and the severe inflammation of the periodontal ligament with destruction of alveolar bone is called periodontal disease.[1] A susceptible host, the presence of pathogenic species, and the absence of so-called "beneficial bacteria".[2] It is generally accepted that the oral biofilm in association with anaerobic bacteria is the main etiological factor in periodontal disease.[3,4] The oral biofilm consists mainly of microbes and host proteins that adhere to teeth within minutes of a dental oral hygiene procedure. Most of these microorganisms can produce tissue destruction in two ways: A. directly, through invasion of the tissue and the production of harmful substances that induce cell death and tissue necrosis, and B. indirectly, through activation of inflammatory cells which can produce and release mediators that act on effectors, with potent proinflammatory and catabolic activity.[5] Some bacteria also interfere with the normal defense mechanisms by deactivation specific antibodies or inhibiting the action of phagocyte cells.[1] Systemic alterations of the cellular and humoral immune responses to periodontal pathogens among chronic periodontitis patients have been evaluated, including immunosuppression, exaggerated inflammatory cell responses, impaired neutrophils, and antibody production.[6,7] Complement and acute phase proteins play an important role in the initiation of host defenses against local infection. Complement is believed to be important in periodontal infections as well. Actually, the only evidence that speaks completely for a role of complement in periodontal disease is that complement components and derived split products are found in abundance in the crevicular fluid. Cytokines play an important role in the pathogenesis of periodontal diseases. Interactions between different cytokines may help to understand the response of gingiva to inflammation and also progression of gingivitis to periodontitis.[8] The periodontal pathogens are resistant to multiple antimicrobial agents, but also susceptible to others.[9]

2. MATERIALS AND METHODS

Patients

One hundred eighteen samples were examined in the present study. They were referred to the Laboratory of Microbiology, Department of Microbiology, College of Medicine, Tikrit University. Patients were of both sexes (140 males and 40 females). Their ages ranged from
16-69 years old. The selection was done randomly among patients in Tikrit University/college of dentistry/dental teaching hospital. The diagnosis of periodontitis was made by the clinical examination including taking dental, medical and family history from the subjects involved in this study, so patients fulfilling the criteria to be diagnosed as periodontitis. Clinical measurements of periodontal parameters used included dental plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment loss using (graduated William’s periodontal probe). Clinical diagnosis in each case was according to the dentist. The interviews were performed for each patient. The questionnaire included general information about the patient e.g.: name, age, sex, smoking, education, residence and treatment if the patients treated or not treated by antibiotics before sampling.

**Sampling**

Samples were obtained from periodontal pockets after supragingival plaque was removed from the teeth to be collected.\[^{10}\] The supragingival dental plaque was removed with sterile cotton, and the tooth surface was dried with compressed air to prevent contamination with saliva. The exclusion of moisture in the mouth with sterile cotton rolls and subgingival plaque was collected from the most inflamed sites by inserting a sterile paperpoint into the periodontal pocket for 10 seconds , when the pocket depth was from 3-7mm. The sample was mixed with 1 ml thioglycollate broth (transport medium) , sealed tightly to avoid contamination and kept at 4°C\[^{10}\]. Samples were processed within 2 days of collection.\[^{11}\] The control group consisting of 34 healthy person (without periodontal disease).

**Measurement of serum complements C3 and C4 protein in periodontal patients**

Determination of the C3 and C4 protein by radial immunodiffusion (RID) plate containing a specific antibody will form an immuno-complex, visible as a ring around the well.\[^{12}\] The ring diameter is direct proportional to the concentration of the analysed protein. The proportion corresponds to the diffusion time and at the end of 72h the square of diameter will be in linear proportion to the concentration.

**Measurement of serum immunoglobulin’s (IgA,IgM,IgG) in periodontal patients**

Determination of IgA, IgM, and IgG protein by radial immunodiffusion (RID) plate containing a specific antibody formed an immuno-complex, visible as a ring around the well.\[^{12}\] The ring diameter was direct proportional to the concentration of the analyzed protein. The proportion corresponded to the diffusion time and at the end of 72h the square of diameter was in linear proportion to the concentration.
Measurement of serum immunoglobulin (IgE) in periodontal patients

IgE was determined by ST AIA PACK IgE II kit (manufactured by Tosoh, Japan). This technique was designed for in vitro diagnostics used only for the quantitative measurement of immunoglobulin E in human serum using TOSOH AIA system analyzers. The AIA-360 sample carousel holds up to 25 patient samples and 25 test cups at one time. The ST AIA PACK IgE II was a two-site immunoensymometric assay which was performed entirely in the AIA PACK test cups [plastic cups containing lyophilized twelve magnetic beads coated with mouse anti-human IgE monoclonal antibody and 100 µl of mouse anti-human IgE monoclonal antibody (to human IgE) conjugated to bovine alkaline phosphate with sodium azide as preservative]. Human IgE present in the test sample was bound with monoclonal antibody immobilized on a magnetic solid phase and labeled monoclonal antibody in the AIA PACK test cups. The magnetic beads were washed to remove unbound enzyme-labeled monoclonal antibody and then were incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binded to beads was directly proportional to the IgE concentration in the test sampled.

Measurement of serum interleukins IL-4 and IL-10 in periodontal patients

Determination of human interleukins in periodontal patient by quantikine ELISA kit [Human IL-4 and Human IL-10] (Manufactured by R&D system, USA).[13,14] This assay was employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-4 or IL-10 were precoated onto a microplate. Standards and samples were pipetted into the wells, and any IL-4 or IL-10 present in bound by the immobilized antibody. After washed away any unbound substances, an enzyme linked polyclonal antibody specific for IL-4 or IL-10 was added to wells. Washed a substrate solution was added to the wells and colour developed in proportion to the amount of IL-4 or IL-10 bound in the initial step to remove any unbound antibody-enzyme reagent. The color development was stopped and the intensity of the colour was measured.[13,14]

3. RESULTS

Serum immunoglobulins levels of IgA, IgM, IgG, and IgE of periodontal patients

Table 1 shows descriptive statistics for serum immunoglobulins concentration between patients and control groups. Higher serum IgA, IgM, and IgG concentration was noticed among the patients with means 174.4, 124.61, and 1097.16 respectively. The differences of serum IgA, IgM and IgG concentrations between patients and control groups was significant.
The differences of serum IgE concentrations between patients and control groups was not significant (p > 0.05).

Table 1: Descriptive statistics for serum immunoglobulins concentration between periodontal patients and control group

| study groups | Cases | | | | | | Controls | | | |
|--------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|              | No.   | Mean  | SD    | SE    | Median | No.   | Mean  | SD    | SE    | Median |
| IgA          | 30    | 174.4 | 133.53| 24.38 | 139.3  | 30    | 243.13| 140.02| 36.15 | 209.7  |
| IgM          | 30    | 124.61| 98.99 | 18.38 | 94.9   | 30    | 157.71| 51.51 | 13.3  | 159    |
| IgG          | 30    | 1097.16| 275.67| 50.33 | 1010.6 | 30    | 914.39| 95.74 | 24.72 | 910.6  |
| IgE          | 61    | 202.78| 319.04| 40.85 | 81.6   | 30    | 372.44| 727.34| 139.98| 58.1   |

SD: standard deviation ; SE: standard error ; No: number ; IgA: t-test=1.602, df=43 , Mann Whitney U-test (U statistic = 306.5, z score = 2.203585, p value < 0.05) significant ; IgM: Mann Whitney U-test (U statistic = 316.5, z score = 2.451178, p value < 0.05) significant ; IgE: t-test=1.526, df=86, p value= 0.254 (not significant) Mann Whitney U-test (U statistic = 836, z score = -0.008925, p value > 0.05) not significant; IgG: t-test=2.482, df=43, p value= 0.017 (significant).

Serum C3 and C4 levels in periodontal patients

Table 2 shows descriptive statistics for serum complements concentration between patients and control groups. Higher serum C3, and C4 concentration was noticed among the patients with means 93.87 and 13.36 respectively . The comparison of serum C3 and C4 concentrations between patients and control groups was significant (p < 0.5).

Table 2: Descriptive statistics for complements C3 and C4 concentration of periodontal patients and control group.

| Complements | Cases | | | | | | Controls | | | |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|              | No.   | Mean  | SD    | SE    | Median | No.   | Mean  | SD    | SE    | Median |
| C3           | 30    | 93.87 | 32.09 | 5.85  | 98     | 30    | 119.1 | 42.29 | 10.92 | 110.5 |
| C4           | 30    | 13.36 | 4.72  | 0.86  | 13.55  | 30    | 16.45 | 3.95  | 1.02  | 15.1  |

SD: standard deviation ; SE: standard error ; No: number ; C3: t-test= -2.232, df=43, p value= 0.031 (significant); C4: t-test= -2.185, df=43, p value= 0.034 (significant).

Serum IL-4 and IL-10 level in periodontal patients

Table 3 shows descriptive statistics for serum interleukins IL-4 and IL-10 concentration between patients and control groups. Higher serum IL-4 and IL-10 concentration was noticed among the patients with means 73.4 and 7.58 respectively . The comparison of serum IL-4
and IL-10 concentrations between patients and control groups was significant (p< 0.5). The descriptive statistics of the variable immunological parameters were listed in Table 4.

Table 3: Descriptive statistics for IL4 and IL10 concentration between periodontal patients and control group.

<table>
<thead>
<tr>
<th>Interleukins</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
</tr>
<tr>
<td>IL4</td>
<td>33</td>
<td>73.4</td>
</tr>
<tr>
<td>IL10</td>
<td>34</td>
<td>7.58</td>
</tr>
</tbody>
</table>

SD: standard deviation; SE: standard error; No: number; IL10: Mann Whitney U-test (U statistic = 267, z score = 3.963592, pvalue is <0.05) significant; IL4: Mann Whitney U-test (U statistic = 4, z score = -4.480105, pvalue is <0.05) significant.

Table 4. Descriptive statistics for immunoglobulins IgA, IgM and IgG; complements C3 and C4; interleukins IL-10 and IL4 concentrations between periodontal patients and control group

<table>
<thead>
<tr>
<th>Components</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
</tr>
<tr>
<td>C3</td>
<td>30</td>
<td>93.87</td>
</tr>
<tr>
<td>C4</td>
<td>30</td>
<td>13.36</td>
</tr>
<tr>
<td>IgA</td>
<td>30</td>
<td>174.4</td>
</tr>
<tr>
<td>IgM</td>
<td>30</td>
<td>124.61</td>
</tr>
<tr>
<td>IgG</td>
<td>30</td>
<td>1097.16</td>
</tr>
<tr>
<td>IgE</td>
<td>61</td>
<td>202.78</td>
</tr>
<tr>
<td>IL4</td>
<td>33</td>
<td>73.4</td>
</tr>
<tr>
<td>IL10</td>
<td>34</td>
<td>7.58</td>
</tr>
</tbody>
</table>

SD: standard deviation; SE: standard error; No: number.

Table 5: The Correlations between immunoglobulins IgA, IgM, IgE and IgG; complements C3 and C4; interleukins IL-10 and IL4 concentrations in periodontal patients

<table>
<thead>
<tr>
<th>Immunological variables</th>
<th>C3</th>
<th>C4</th>
<th>IgM</th>
<th>IgA</th>
<th>IgG</th>
<th>IgE</th>
<th>IL4</th>
<th>IL10</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>1</td>
<td><strong>0.312</strong></td>
<td>0.071</td>
<td>0.001</td>
<td>-0.242</td>
<td>-0.196</td>
<td>-0.223</td>
<td>0.005</td>
</tr>
<tr>
<td>C4</td>
<td><strong>0.312</strong></td>
<td>1</td>
<td>-0.054</td>
<td><strong>0.370</strong></td>
<td>0.024</td>
<td>0.044</td>
<td>-0.168</td>
<td><strong>-0.357</strong></td>
</tr>
<tr>
<td>IgM</td>
<td>0.071</td>
<td>-0.054</td>
<td>1</td>
<td>0.119</td>
<td>-0.100</td>
<td>0.149</td>
<td>-0.026</td>
<td>-0.107</td>
</tr>
<tr>
<td>IgA</td>
<td>0.001</td>
<td><strong>0.370</strong></td>
<td>0.119</td>
<td>1</td>
<td>0.002</td>
<td>0.149</td>
<td>-0.102</td>
<td>-0.066</td>
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<tr>
<td>IgG</td>
<td>-0.242</td>
<td>0.024</td>
<td>-0.100</td>
<td>-0.002</td>
<td>1</td>
<td>-0.102</td>
<td>0.149</td>
<td>-0.194</td>
</tr>
<tr>
<td>IgE</td>
<td>-0.196</td>
<td>0.044</td>
<td>0.149</td>
<td>0.149</td>
<td>-0.102</td>
<td>1</td>
<td>-0.265</td>
<td>-0.202</td>
</tr>
<tr>
<td>IL4</td>
<td>-0.223</td>
<td>-0.168</td>
<td>-0.026</td>
<td>-0.102</td>
<td>0.149</td>
<td>-0.265</td>
<td>1</td>
<td>0.212</td>
</tr>
<tr>
<td>IL10</td>
<td>0.005</td>
<td><strong>-0.357</strong></td>
<td>-0.107</td>
<td>-0.066</td>
<td>-0.194</td>
<td>-0.202</td>
<td>0.212</td>
<td>1</td>
</tr>
</tbody>
</table>

*. Correlation is significant at the 0.05 level (2-tailed).
The correlation between different immunological variables in periodontal patients

Table 5 shows the correlation between different immunological variables in periodontal patients. It was found that a significant positive linear correlation between C3 and C4, C4 and IgA, and a significant negative correlation between C4 and IL10.

4. DISCUSSION

The present study showed that the mean serum level of IgG was significantly higher in patients with severe periodontitis as compared with healthy control groups as showed in Table 1. These findings were almost similar to other studies reported by Novo et al and Galaviz et al who reported that 57% of periodontitis patients were positives IgG.[15,16] The involvement of host response to bacteria involved in periodontal disease can be detected by the serum immunoglobulins to particular bacteria and/or their antigens. The current study showed that severe periodontitis patients had a significant elevation of the serum IgG, IgA, and IgM levels as compared to healthy control. The results found here were almost similar to those of Califano et al, Engstrom et al, and Kobayashi et al who mentioned that the elevation in IgG level as a result of host response to bacterial colonization.[17,18,19] Similarly, Quinn et al found that IgG antibodies have been considered important in preventing periodontal destruction in patients with aggressive and chronic periodontitis.[20] On the other hand, Craig et al reported that the serum IgG antibody may be reflective of the destructive periodontal disease, and its level can be considered a risk indicator for disease progression.[21]

The production of antibodies against self structures could be involved in the pathogenic mechanism of periodontitis. These results showed a similarity with those of Moor et al, Ogrendik et al, Honmal et al, Mican and Kenichi et al who found that IgG, IgM and IgA were highly significant in periodontal patients.[22,23,24,25,26] Parthiba, Kaslick et al, and Ranney et al found that serum IgG levels is significantly higher than the levels in controls (p<0.007) but serum IgA (p<0.020) and the IgM (p<0.0001) was significantly higher than the levels in controls.[27,28,29] The elevation in serum IgG levels may be due to the increased antibody production to neutralize bacterial toxins. Anil et al reported an increased IgA content of the inflamed gingival, which may be the reason for the increased levels of IgA.[30]

The increased levels of serum IgM may reflect a response to the gram negative bacteria which are most commonly associated with periodontitis.[27] The increased levels of IgG in saliva of patients with aggressive periodontitis may be due to the predominating synthesis of IgG and the transfer of this and serum-derived IgG from the gingival tissue to the oral cavity. Prolonged antigenic stimulus in an infectious condition like periodontal disease may also
stimulate the local IgA immune system. Elevated IgM levels may be due to the raised level of glandular secretory output or from increased leakage from the blood via the gingival sulcus.\\(^{[27]}\\)

The present results showed highly significant differences in the serum C3 and C4 between periodontal patients and control as shown in Table 2. These results were almost similar to those of Moor et al., Ogrendik et al., Honmal et al., Noor et al., Michae and Kenichi et al. who found that C3 and C4 were highly significant in periodontal patients more than control.\\(^{[22,31,32,33,34,35]}\\) Ken and Shinkein found that periodontal inflammation increases with a concomitant increase in complement level of C3 and C4 up to 85%.\\(^{[36,37]}\\) Riina found that C3 and C9 components of complement were observed deposited on the basement membranes of both control and gingival epithelium of periodontal patients.\\(^{[38]}\\) Serum C3 showed highly significant differences between patients and control.\\(^{[39]}\\) In contrast, Harvey found that no differences between the concentration of C3, C4, and C5 in periodontal patients and control.\\(^{[40]}\\) Furthermore, Harvey proposed a model for the pathogenesis of chronic periodontal disease based upon the capacity plaque bacteria to activate the alternative complement pathway. Hawly and Falkler demonstrated that sera from individuals undergoing periodontal therapy contained IgM antibodies that could bind to LPS from Leptotrichia buccalis and activate complement. The same authors demonstrated the capacity of cell wall components and particularly lipopolysaccharide of Fusobacterium nucleatum to activate the alternative complement pathway in serum. Moreover, Tsai demonstrated that some Gram-positive bacteria including A. viscosus, Streptococcus mutans, and S. sanguis, could activate both the classical and alternative complement pathways in human sera.\\(^{[41]}\\) Activation of complement by Propionibacterium acnes and Bacteroides gingivalis was reported by Harvey.\\(^{[39]}\\) They further showed that P. acnes activity was mainly in the cell wall, while B. gingivalis activated complement via its LPS. Also LPS preparations from B. oralis, F. nucleatum, and Veillonella parvula for their ability to induce C3 cleavage in human serum, and found that the classical (F. nucleatum and V. parvula) or alternative pathway (B. oralis) were responsible for C3 conversion, and in a survey of the complement-activating capacity of dental plaque samples and a variety of bacteria, it was shown by Okuda and Takazoe that several species of Actinomyces, Streptococcus, Fusobacteria, and Veillonella, as well as P. acnes and Selenomonas sputigena activate complement.\\(^{[42]}\\) Both pathways could be shown to be activated. Bolton and Dyer demonstrated complement activation by Capnocytophaga sp. by quantitating the appearance of immunoreactive C3a in serum.\\(^{[43]}\\)
The host defense system and the cells of the periodontium are all linked by complex processes in which soluble mediators and cytokines coordinate tissue turnover, inflammatory processes and immune response. The present study showed that the serum level of pro-inflammatory cytokine IL-4 and IL-10 were significantly higher in patients with chronic periodontitis as compared with healthy control group (Table 3). Zahraa et al found that significant decrease serum of IL-10 level in chronic periodontal patients (0.039pg /ml) as compared to healthy control (0.095pg /ml) which is statistically significant (p<0.01). Also Passoja et al and Goutoudi found a significant decrease of IL-10 in serum of periodontal patients compared to healthy control. Bodet et al reported that there was no significant differences in the level of serum IL-10 between periodontal patients and healthy group. Furthermore, Maryam et al found that the level of IL-4 increased significantly in control group in comparison with the test group (p=0.002). Pradeep et al reported that the mean concentration of IL-4 decreased from periodontal health to disease. Giannopoulou et al have also shown that IL-4 was higher in the periodontally healthy group, but very low in the periodontal disease group. Moreover, Nadhi et al found that IL-4 was statistically significant among patients and control. Because periodontitis was a complex disease, the mere presence of putative pathogens do not imply that disease was or will be present, but high numbers of organisms are most likely required. Moreover, the increased content of some bacteria in dental plaque of patients with periodontitis is associated with increased levels of proinflammatory cytokines, such as TNF-α, which was associated with the enhanced dental plaque loaded with periodontal pathogens. However, Livia et al found that IL4 was associated with increased level of periodontal pathogenic bacteria.

5. CONCLUSIONS

The immunological analysis results showed that the comparison of serum IgA, IgM and IgG; C3 and C4; IL-4 and IL-10 concentrations between patients and control groups were significant (p <0.05). It was found significant positive linear correlation between C3 and C4, C4 and IgA, and significant negative correlation between C4 and IL-10(p<0.05). The present results showed highly significant differences in the serum C3, C4,IgA,IgM,IgG,IL-4 and IL-10 between periodontal patients and control. The levels of these immunological components were higher in patients sera than controls.
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