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CYTOKINE LEVELS IN PATIENTS WITH BRUCELLOSIS AND THEIR RELATIONS WITH THE TREATMENT

*H Akbulut, I Celik, A Akbulut

Abstract

Purpose: To determine the serum levels of proinflammatory and some of the Th1/Th2 cytokines in brucellosis and their alterations with treatment and outcome. Methods: Twenty-eight acute and seven subacute brucellosis patients diagnosed clinically were included in the study. Twenty healthy volunteers were also included. Brucella standard tube agglutination tests and blood culture were conducted on all subjects. Cytokine levels of pre- and post-treatment period serum samples were measured by ELISA. Results: The mean serum levels of IL-6, IFN-γ and TNF-α were significantly higher in brucellosis patients compared to the control group (P < 0.05). No significant differences were found between patient and control groups in terms of IL-1β, TGF-β1, IL-2, IL-4 and IL-8 levels. There was a positive correlation between IFN-γ, TNF-α and IL-6 levels with CRP levels. IL-6, IFN-γ and TNF-α levels measured after treatment were statistically significantly lower than pre-treatment values (P < 0.001). No differences were found in the levels of these cytokines between acute and subacute patients’ sera. IL-6, IFN-γ and TNF-α levels were higher in acute or subacute brucellosis patients. Conclusions: Although the levels of the cytokines were decreased significantly with effective and adequate treatment these alterations did not correlate with the extent or activity of the disease.

Key words: Brucellosis, cytokines, treatment

Brucella is a facultative, intracellular pathogen that can reside within phagocytic cells (macrophages) of the host and is apparently resistant to the normal mechanisms of bacterial killing.¹ The response against Brucella spp. involves the whole gamut of the immune system, from innate to adaptive immunity.² Cytokines appear to have an important role in the pathogenesis of brucellosis and the Th1/Th2 balance may be involved in the susceptibility or resistance to the disease.³,⁴ Th1 cells are mediators of the effector mechanisms required for resistance to intracellular pathogens, while a Th2 cell response is detrimental in combating this type of infection.⁵ A Th1 response is essential for resolution of the primary infection caused by Brucella and the essential aspect of this response appears to be IFN-γ production.⁶ IFN-γ is required for elimination of Brucella and is required for host survival in the face of virulent Brucella challenge.⁷,⁸ Th2 cytokine, IL-4, evoke strong antibody responses and eosinophil accumulation; nevertheless inhibit several functions of phagocytic cells.⁹ Both CD4⁺ and CD8⁺ T cell populations contribute to immune response to B. abortus producing IFN-γ and IL-2. B. abortus can induce differentiation of Th0 into Th1-type cell.¹⁰

The aim of this study was to investigate the levels of serum IL-1β, IL-6, IL-8, TNF-α, TGF-β1, Th1 (interferon-γ, IL-2) and Th2 cytokines (IL-4) and their alterations with treatment in patients with acute and subacute brucellosis.

Materials and Methods

Patients

The study was performed between December 2004 and April 2006. Patients were grouped as acute, subacute and chronic brucellosis according to duration of the disease: <8 weeks, 8-52 weeks or >52 weeks, respectively.¹¹ The study included a total of 28 acute brucellosis cases (12 males and 16 females; 36.43 ± 18.55 years) and 7 subacute cases (4 males and 3 females; 34.43 ± 23.31 years). The diagnostic criteria were (a) isolation of a Brucella species from blood cultures (Becton Dickinson 9050, USA) and/or (b) the finding of ≥1/160 antibody titer to Brucella by standard tube agglutination (STA) method in the presence of a compatible clinical picture including acute or insidious onset of fever, night sweats, undue fatigue, anorexia, weight loss, headache and arthralgia or a clinically compatible case that was epidemiologically linked to a confirmed case or that had supportive serology (i.e., Brucella agglutination titer of greater than or equal to 160 in one or more serum specimens obtained after onset of symptoms).¹² Ten millilitres of peripheral blood were taken from each patient and inoculated into Bactec blood culture bottles and incubated in the Bactec 9050 System (Becton Dickinson, Sparks, MD, USA) until a positive signal was observed. Subcultures were obtained on sheep blood agar, chocolate agar and eosin methylene blue (EMB) agar and incubated at 35 °C in a 5% CO₂ atmosphere for at least 48 h. Small and whitish colonies appeared only on blood and chocolate agar after 48 h and no growth was observed on EMB agar. The colonies that yielded gram negative cocobacilli and were positive for the oxidase and urease tests were identified as Brucella spp. Brucella species
were isolated from the blood cultures in 18 cases (51.4%). All isolated Brucella species were identified as Brucella melitensis by specific PCR in Microbiology Department. All patients were treated with combination of doxycycline, 100 mg twice a day orally for 45 days or tetracycline hydrochloride, at doses of 0.5 g every 6 h for 45 days with one of the rifampicin, 600 mg/day in a single morning dose for 45 days 1 g/day intramuscularly for 21 days, according to the World Health Organization recommendations. All of the patients (n = 35) adjusted to the treatment and completely recovered clinically at the end of the treatment.

Control group

The control group included 20 volunteers, 10 of them male and 10 of them female, ages ranging between 18 and 60 (36.6 ± 17.8) years. The cases in the control group were STA negative, showed erythrocyte sedimentation rate (ESR) within normal limits and did not have any complaints. Exclusion criteria for the healthy control subjects included smoking, medication, pregnancy and any abnormalities in renal and liver function tests. The consent for this study was approved by Local Ethics Committee. All patients and healthy individuals were informed about the study and informed consent was taken from all.

Determination of cytokine levels

Venous blood samples (5 mL) were taken from all patients (before and after the treatment) and controls. The blood samples were centrifuged at 5000 rpm for 10 min and sera collected were stored at –80 °C until assayed. IL-1β, IL-6, IL-8, TNF-α, TGF-β1, Th1 cytokines (interferon-γ, IL-2) and Th2 cytokines (IL-4) levels in all serum samples were measured at the same time by ELISA method. A commercial kit was used for this purpose and the study was performed according to the kit procedure (Medgenix, Biosource International, Camarillo, USA). CRP levels (immunoturbidometric technique, Schiapparelli Biosystems, Netherlands) before and after the treatment were also measured.

Statistical analysis

Statistical analyses were made using SPSS 11.0 for Windows. Wilcoxon’s Signed Rank Test was used for evaluations within groups and Mann-Whitney-U Tests were used for comparisons between groups. Correlations between parameters were evaluated with Spearman correlation analysis. P < 0.05 values were accepted as statistically significant.

Results

The median of Brucella STA and 2-ME test levels were 1/320 in patients. Epidemiological data, complaints and laboratory findings of the patients are shown on Tables 1 and 2, respectively. Mean serum cytokine levels of patients in the pre-treatment and post-treatment and of healthy control group are presented in Table 3. It was found that the mean serum levels of IL-6, IFN-γ and TNF-α (mean ± SD, 15.7 ± 2.7, 12.9 ± 2.9 and 53.3 ± 10.1 pg/mL, respectively) were significantly higher in all brucellosis patients compared to the control group (mean ± SD, 1.1 ± 0.8, 6.9 ± 1.3 and 8.4 ± 1.2 pg/mL, respectively) (P < 0.05). However, no significant differences were found between patient and control groups in terms of IL-1β, TGF-β1, IL-2, IL-4 and IL-8 levels (P > 0.05), there was a positive correlation between pre-treatment IFN-γ, TNF-α and IL-6 levels and CRP (r: 0.494, 0.624, 0.846, 0.583, respectively; P < 0.05). Mean serum levels of IL-6, IFN-γ and TNF-α measured at the end of the treatment were statistically significantly lower than pre-treatment values (P < 0.001). The levels of other cytokines did not show any statistically significant differences between pre- and post-treatment period (P > 0.05).

Furthermore, no differences were found in the levels of these cytokines between acute and subacute patients with brucellosis. Mean serum levels of cytokines in patients with acute and subacute brucellosis and the control group are presented in Table 2. Cytokine levels in patients with positive blood cultures did not show statistically significant differences compared to the ones with negative culture. No statistically significant differences were observed in terms of IL-1β, IL-2, IL-4 and IL-8 levels between pre- and post-treatment period. No correlation was observed in post-treatment levels of IFN-γ, TNF-α, IL-6 and CRP levels (Table 4).

Table 1: Epidemiologic and clinical features of patients with brucellosis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.6 ± 1.8</td>
</tr>
<tr>
<td>Haematocrite (%)</td>
<td>36.7 ± 5.2</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (mm/h)</td>
<td>37.75 ± 29.7</td>
</tr>
<tr>
<td>C-reactive protein (g/dL)</td>
<td>33.53 ± 27.23</td>
</tr>
<tr>
<td>White blood cell count (cell/mm³)</td>
<td>6888 ± 2647</td>
</tr>
</tbody>
</table>

Table 2: Physical and laboratory findings of patients with brucellosis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingesting raw milk/fresh cheese</td>
<td>60.0</td>
</tr>
<tr>
<td>Ingesting unpasteurized fresh butter</td>
<td>20.0</td>
</tr>
<tr>
<td>Ingesting raw meat balls</td>
<td>36.7</td>
</tr>
<tr>
<td>Fever</td>
<td>85</td>
</tr>
<tr>
<td>Sweating</td>
<td>72.5</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>65</td>
</tr>
<tr>
<td>Headache</td>
<td>52.5</td>
</tr>
<tr>
<td>Malaise</td>
<td>65</td>
</tr>
<tr>
<td>Lack of appetite (anorexia)</td>
<td>50</td>
</tr>
<tr>
<td>Back pain</td>
<td>17.5</td>
</tr>
<tr>
<td>Weight loss</td>
<td>45</td>
</tr>
</tbody>
</table>

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Table 3: Mean serum cytokines and CRP levels of patients in the pre- and post-treatment and of healthy control group

<table>
<thead>
<tr>
<th>Cytokines (pg/mL) and CRP</th>
<th>Patients (n = 35)</th>
<th>Control group (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-treatment</td>
<td>Post-treatment</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>12.9 ± 2.9*</td>
<td>6.7 ± 1.89</td>
</tr>
<tr>
<td>IL-2</td>
<td>0.76 ± 0.34</td>
<td>1.65 ± 1.36</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.61 ± 0.61</td>
<td>2.26 ± 1.13</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.55 ± 0.33</td>
<td>2.35 ± 0.55</td>
</tr>
<tr>
<td>IL-6</td>
<td>15.7 ± 2.7*</td>
<td>5.10 ± 1.93</td>
</tr>
<tr>
<td>IL-8</td>
<td>25.87 ± 5.72</td>
<td>21.48 ± 4.79</td>
</tr>
<tr>
<td>TNF-α</td>
<td>53.3 ± 10.1*</td>
<td>23.28 ± 11.53</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>432.4 ± 103.7</td>
<td>381.5 ± 95.1</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>32.33 ± 4.24*</td>
<td>7.6 ± 1.1</td>
</tr>
</tbody>
</table>

*vs. control and post-treatment (P < 0.05)

Table 4: Mean serum cytokine levels in patients with acute and subacute phases of brucellosis and in healthy controls

<table>
<thead>
<tr>
<th>Cytokines (pg/mL)</th>
<th>Patients (n = 35)</th>
<th>Control group (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute (n = 28)</td>
<td>Subacute (n = 7)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>13.39 ± 3.08*</td>
<td>11.37 ± 1.04*</td>
</tr>
<tr>
<td>IL-2</td>
<td>0.85 ± 0.41</td>
<td>0.38 ± 0.18</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.02 ± 0.49</td>
<td>2.95 ± 2.23</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.68 ± 0.40</td>
<td>1.03 ± 0.40</td>
</tr>
<tr>
<td>IL-6</td>
<td>15 ± 2.91*</td>
<td>18.62 ± 7.33*</td>
</tr>
<tr>
<td>IL-8</td>
<td>24.48 ± 5.71</td>
<td>31.42 ± 18.28</td>
</tr>
<tr>
<td>TNF-α</td>
<td>53.20 ± 10.93*</td>
<td>53.87 ± 27.15*</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>416.4 ± 100.4</td>
<td>481.5 ± 109.7</td>
</tr>
</tbody>
</table>

*vs. control (P < 0.05)

Discussion

In our study, it was found that the mean serum levels of IL-6, IFN-γ and TNF-α were statistically significant and high in acute and subacute brucellosis cases, in comparison to the control group (P < 0.05). The importance of cytokine responses in the pathogenesis of brucellosis have previously been studied in animal models. The real numbers, functions and interactions amongst one another of hundreds cytokines and cytokines like molecules synthesized in human body are not actually known. To determine the complex cytokine profile during an infectious disease, it is an important constituent to understand the pathogenesis, prognosis and treatment of the disease. Facultative intracellular bacteria, including Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium leprae, Brucella abortus and Salmonella spp., survive within normal resident macrophages and other non-professional phagocytes. Th1-dominated immune responses predominantly produce a phagocyte-dependent inflammation. Th2 cells evoke strong antibody responses, including IgE. Th1 and Th2 cells depressed each other. Meanwhile, immune responses turn from one type to another.

Pasquali et al., concluded that IL-4 was never detected in B. abortus RB51-vaccinated mice. However, they detected a very low level of IL-4 after challenge infection with B. abortus 2308 cells. In a limited number of studies, involving acute brucellosis patients, IL-1 and IL-4 were reported to be at undetectable levels in the serum, while IL-2 and IFN-γ levels were significantly higher in brucellosis patients than they were in the control group. It has been reported that pre-treatment CD3 + IFN-γ + levels were higher in responsive patients than the unresponsive group. In addition to the studies showing induction of Th1-type immune response by brucellosis; Galanakis et al., have reported that in children with brucellosis, serum IL-4 levels increased in the acute phase of the disease. In our study, IL-1 β and IL-4 levels were not statistically different between patient and control groups.

In vitro experiments have demonstrated that B. abortus induces human monocytes to secrete the proinflammatory cytokines. Refik et al., have been found that patients with brucellosis had significantly elevated serum levels of IL-6 and IL-8 compared to healthy controls but levels of TNF-α were not different. Some studies have suggested that Brucella strains did not induce TNF-α in human macrophages. On the contrary, in our study TNF-α and IL-6 levels were found to be higher in the patients compared with the values.
of post-treatment and the control group. Since IL-6 plays an important role in inflammation, it can be suggested that IL-6 takes place in the process and pathogenesis of brucellosis. It was found that TNF-α correlated with the increase in IFN-γ levels. Giambartolomei et al.,18 demonstrated that patients with acute brucellosis display a Th1 type response with cell proliferation and production of IFN-γ and IL-12, whereas patients with the chronic form (7 of 11 non-responders) of the disease do not. We did not found any statistically significant difference in the levels of IL-6, IFN-γ and TNF-α between acute and subacute cases.

In conclusion, our results would suggest a definite role of IFN-γ and proinflammatory cytokines in the pathogenesis of brucellosis. However, it was observed that these alterations did not correlate with the extent or activity of the disease.

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