Acute Phase Proteins in Human Brucellosis


Acute phase proteins namely alpha-1-antitrypsin and ceruloplasmin were estimated together with the qualitative detection of C-reactive protein in patients suffering from acute brucellosis as diagnosed by their clinical signs, symptoms and serology. The levels of acute phase proteins were compared with that of the conventional direct agglutination test and the serum levels of immunoglobulin classes IgG, IgA and IgM and various markers of acute inflammation—namely C3, C4 components of the complements and albumin. A positive correlation was observed between the levels of acute phase reactant proteins, immunoglobulin levels and the titre of the Brucella-specific antibodies. However, a negative correlation ($r = -0.86; p < 0.05$) was observed between serum albumin levels and that of the acute phase proteins as expected. Upon following up the patients for 5 weeks no significant differences were observed in the concentrations of the immunoglobulins and the specific antibody titre while the levels of alpha-1-antitrypsin and ceruloplasmin decreased significantly ($p < 0.05$) and the C-reactive protein was found to be negative.

Human brucellosis is the most frequently observed infectious disease in Saudi Arabia. It is a localized as well as a generalized infection with systemic manifestations. The diagnosis is often difficult because of the problems in obtaining a positive culture, which is the only absolute diagnosis for brucellosis. Therefore, much reliance has been placed on serology. The nature of humoral immune response and the class of specific antibody involved at a particular stage of the disease is subject to much debate. It has been demonstrated that all the three major classes of immunoglobulins are involved at some time during the course of infection. However, with the advent of more sophisticated techniques it is possible to detect the individual classes of Brucella-specific antibodies.

An increase in specific IgM is considered as a marker of acute infection, while a continuous rise in specific IgG indicates chronic infection. However, the detection of specific IgM and IgG antibodies for the staging of Brucella infection...
pose a problem in an average routine clinical laboratory because of the limitation in the use of sophisticated techniques. This paved the way for us to look for the possible markers for the laboratory diagnosis of acute brucellosis. Therefore, it has been proposed that the measurement of acute phase proteins (APP) may have some significance in supporting the diagnosis of acute brucellosis.

The available data on acute phase proteins in brucellosis is scanty. The present report is an attempt to compare the serological response of acute brucellosis with the levels of acute phase proteins—namely, alpha-1-antitrypsin, ceruloplasmin and albumin and correlate them with the concentrations of immunoglobulins IgG, IgA, IgM and C3, C4 components of complement.

Materials and Methods

Sixty patients attending the outpatient clinic at King-Fahd Specialist Hospital, Buraidah and provisionally diagnosed to be suffering from acute brucellosis based on clinical signs and symptoms were selected for the study. Patients reported to be suffering from other bacterial, viral or protozoal infections were excluded. Accordingly, there were 31 males and 29 females with an average age of 41 years. Sixty age- and sex-matched individuals, mostly blood donors and laboratory personnel not reported to be suffering from any kind of infection were selected as controls. None of the females in the test or the control group was pregnant.

Blood was cultured for Brucella wherever possible. The clinical diagnosis was confirmed serologically using a conventional Brucella agglutination test. Patients showing a titre of 1:160 or more were considered for study. Care was taken to exclude the prozone phenomenon using the anti-globulin test. C-Reactive protein was determined qualitatively using the standard latex agglutination method. Other acute phase proteins—namely, alpha-1-antitrypsin and ceruloplasmin were assayed by the single radial immunodiffusion method of Mancini, using the commercially available radial immunodiffusion plates (Behring, Germany) containing monospecific antiseraum against the protein being estimated. We introduced 5 μl of serum into the wells of the immunodiffusion plates and allowed it to diffuse for a period of 48 hours; then the diameters of the precipitates were measured using a radial immunodiffusion plate reader and the concentrations corresponding to the diameters were recorded from the table of calibration values provided with the kit. In an aliquot of each serum sample the levels of complement components C3 and C4, immunoglobulins IgG, IgA and IgM were measured using the method of Mancini. The levels of total proteins, albumin and globulins were estimated using an autoanalyser (Hitachi, 704). A second sample was obtained in 20 patients after 5 weeks of treatment and the above-mentioned investigations were repeated. The data were analysed using ‘Student’s’ t test.

Results

The results are shown in Tables 1 and 2. The Brucella antibody titre ranged between 1:160 and 1:5120. A titre of 1:320 was observed frequently (56.6%). No relation was observed between the antibody titre and age, sex or clinical picture. C-Reactive protein was positive in 50% of the cases, which is in agreement with an earlier study. The levels of total IgG, IgM, IgA, the complement components C3, C4 and the acute phase proteins alpha-1-antitrypsin and ceruloplasmin in patients and controls. A significant difference was observed between the two groups in all the parameters studied. A significant correlation was observed between IgG and IgM (r = 0.58; p < 0.001) as also between IgG and IgA (r = 0.51; p < 0.001). The levels of C4 correlated significantly with those of

<table>
<thead>
<tr>
<th>Subjects</th>
<th>IgG (mg/dl)</th>
<th>IgM (mg/dl)</th>
<th>IgA (mg/dl)</th>
<th>C3 (mg/dl)</th>
<th>C4 (mg/dl)</th>
<th>Alpha-1-antitrypsin (mg/dl)</th>
<th>Ceruloplasmin (mg/dl)</th>
<th>Albumin (A) (g/dl)</th>
<th>Globulin (G) (g/dl)</th>
<th>A/G ratio</th>
<th>Total Protein (g/dl)</th>
</tr>
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<tbody>
<tr>
<td>Patients (Mean ± SD)</td>
<td>1587.68</td>
<td>221.10</td>
<td>350.07</td>
<td>124.16</td>
<td>46.98</td>
<td>634.07</td>
<td>93.69</td>
<td>3.94</td>
<td>2.92</td>
<td>1.41</td>
<td>6.87</td>
</tr>
<tr>
<td>Controls (Mean ± SD)</td>
<td>1211.76</td>
<td>126.63</td>
<td>277.73</td>
<td>108.12</td>
<td>38.65</td>
<td>426.35</td>
<td>63.63</td>
<td>4.69</td>
<td>2.65</td>
<td>1.87</td>
<td>7.33</td>
</tr>
<tr>
<td>t Value</td>
<td>3.60</td>
<td>4.62</td>
<td>2.21</td>
<td>2.34</td>
<td>2.46</td>
<td>4.36</td>
<td>9.01</td>
<td>6.85</td>
<td>2.13</td>
<td>4.83</td>
<td>2.59</td>
</tr>
</tbody>
</table>

Significance: p < 0.001 p < 0.001 p < 0.005 p < 0.025 p < 0.025 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.05 p < 0.001 p < 0.025

Number in parentheses indicates sample number.
Table 2

<table>
<thead>
<tr>
<th>Subjects</th>
<th>IgG (mg/dl)</th>
<th>IgM (mg/dl)</th>
<th>IgA (mg/dl)</th>
<th>C3 (mg/dl)</th>
<th>C4 (mg/dl)</th>
<th>Alpha-1-antitrypsin (mg/dl)</th>
<th>Ceruloplasmin (mg/dl)</th>
<th>Albumin (A) (g/dl)</th>
<th>Globulin (G) (g/dl)</th>
<th>A/G ratio</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(Before treatment)</td>
<td>1756 ± 237.80</td>
<td>433.20</td>
<td>124.50</td>
<td>49.84</td>
<td>659.0</td>
<td>94.99</td>
<td>3.90</td>
<td>3.13</td>
<td>1.35</td>
<td>7.01</td>
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<tr>
<td>(20)</td>
<td>± 93.07</td>
<td>± 198.0</td>
<td>± 281.70</td>
<td>± 55.62</td>
<td>± 25.91</td>
<td>± 294.50</td>
<td>± 31.27</td>
<td>± 0.70</td>
<td>± 1.11</td>
<td>± 0.39</td>
<td>± 1.39</td>
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<tr>
<td>Mean ± SD</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(After treatment)</td>
<td>1553 ± 250.20</td>
<td>323.30</td>
<td>106.00</td>
<td>39.20</td>
<td>429.10</td>
<td>73.99</td>
<td>4.36</td>
<td>2.85</td>
<td>1.65</td>
<td>7.22</td>
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<tr>
<td>(20)</td>
<td>± 728.6</td>
<td>± 224.60</td>
<td>± 165.70</td>
<td>± 39.31</td>
<td>± 19.69</td>
<td>± 157.70</td>
<td>± 24.79</td>
<td>± 0.58</td>
<td>± 0.86</td>
<td>± 0.46</td>
<td>± 0.76</td>
</tr>
<tr>
<td>t Value</td>
<td>1.07</td>
<td>0.13</td>
<td>1.61</td>
<td>1.29</td>
<td>2.31</td>
<td>2.24</td>
<td>2.23</td>
<td>2.24</td>
<td>2.06</td>
<td>3.07</td>
<td>0.66</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>NS</td>
<td>p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Number in parentheses indicates sample number.
NS indicates statistically not significant.
p indicates level of statistical significance.

C3 (r = 0.35; p < 0.001) and also with IgG (r = 0.58; p < 0.001) and IgA (r = 0.31; p < 0.001). The levels of the Brucella antibodies and immunoglobulin profiles were in agreement with earlier studies.4,6,7,10,11

Alpha-1-antitrypsin, ceruloplasmin and globulin levels in the patient group were significantly higher than those in the control group, while the levels of serum albumin and total proteins were significantly lowered in the patients when compared with controls. A significant correlation was observed between the concentrations of ceruloplasmin and alpha-1-antitrypsin (r = 0.36; p < 0.001) as also albumin (r = −6.86; p < 0.05). On the other hand, the levels of serum albumin correlated with the total protein (r = 0.28; p < 0.001) and globulins (r = 0.69, p < 0.001).

Table 2 shows the results of the study in 20 cases before and after 5 weeks follow-up. No significant differences were observed in the specific antibody titre and the concentrations of IgG, IgA, IgM and C3 levels after follow-up, while the concentrations of C4, alpha-1-antitrypsin and ceruloplasmin decreased significantly after 5 weeks. The levels of globulins and total proteins remained unchanged. On the other hand C-reactive protein was found to be negative upon follow-up.

Discussion

This study has thrown light on the measurement of acute phase proteins (APP) as markers to support the diagnosis of acute brucellosis. The APP are a group of glycoproteins synthesized in the liver and their concentration in the plasma increases as a result of tissue damage and inflammation.12 APP play a major role as mediators of inflammatory process. Their synthesis is stimulated by prostaglandins or macrophage derived factors—namely interleukin 1 and interleukin 6 which are released from the damaged tissue at the site of inflammation.13

In acute inflammation or tissue damage C-reactive protein rises rapidly within the first 12 hours, alpha-1-antitrypsin and ceruloplasmin rise 24–72 hours after the onset of inflammation.14 These events are followed by a moderate rise in C3 and C4 after 4–5 days, wherein the complement behaves as an acute phase reactant. The increase in the levels of the APP is associated with a decrease in albumin concentration. Measurement of APP is of considerable value in the detection, prognosis and therapeutic monitoring of patients with tissue damage.14 However, to our knowledge, studies on APP in brucellosis have not been documented. The present report indicates that in brucellosis APP synthesis is stimulated, reaching a peak and gradually falling by 5 weeks possibly due to resolution, while no change was observed in the antibody titre for that duration indicating that a period of 5 weeks is not sufficient to observe any significant changes in the antibody titre. The changes in level of acute phase proteins and albumin in brucellosis could be due to their production locally at the site of inflammation with a spill-over into the blood. The increased levels of C3 and C4 is attributed to the acute inflammatory process and ensuing tissue damage. The endotoxaemia, phagocytic stimuli and other events associated with inflammatory response of brucellosis may increase the synthesis of the complement components, C3 and C4.

The observed increased levels of IgM, IgG, and IgA are due to the stimulation of humoral immune responses by the Brucella antigens.

A positive correlation has been observed between the Brucella antibody titre, immunoglobulin
levels and the concentration of the APP possibly reflecting the intensity of stimulation or the extent of the inflammatory response.

The results indicate that the measurement of APP may have some diagnostic significance in the laboratory diagnosis and response to treatment of acute brucellosis along with the measurement of specific IgM antibodies for the delineation of early stage infections and subclinical cases, especially in the endemic areas. The APP measurements are simple and can be performed even in a small routine clinical laboratory with limited facilities.

Acknowledgements

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References