LEVELS OF SERUM IMMUNOGLOBULINS AND COMPLEMENTS IN PATIENTS WITH VISCERAL LEISHMANIASIS

Wafaa Sadoon Shani

Department of Biology, College of Science, University of Basrah, Basrah, Iraq.

(Received 11 January 2006, Accepted 26 April 2006)

Key words: IgG, leishmaniasis, heteroxenous.

ABSTRACT

Forty sera of patients infected with *Leishmania donovani* and (10) sera from apparently healthy subjects were tested by single radial immunodiffusion to visualize the concentration of IgG, IgM, IgA, C3 and C4. Recent results showed that there was a highly significant increase in the level of IgG, IgM, C3 and C4 but there were no significant differences in the level of IgA between the two-studied groups.

INTRODUCTION

*Leishmania donovani* is an obligate intracellular parasite that infects macrophage of the vertebrate host resulting in visceral leishmaniasis in humans, which is responsible for morbidity and mortality in many humans throughout the world (1).

This protozoan has a heteroxenous life cycle, living first as an intracellular amastigote in the vertebrate host, then as a motile flagellated promastigote in the gut lumen of the sand fly vector (2). Infective promastigote entering the blood of vertebrate is covered by two key molecules: the protein gp 63 and a lipophosphoglycan. Both of these molecules mediate the uptake of promastigote by interacting with components of the complement system and with surface molecules on the macrophage (3).

Clinically *L. donovani* infection may range from asymptomatic to progressive, fully developed Kala-azar. The immunology of *L. donovani* has not been studied to the extent that *Leishmania major* has, at least in mice (4). Beside the presence of cases infected with this parasite in Basrah hospitals, as the aim of the present study is to throw some light on the immune state of patients with kala-azar, especially a humoral part of immunity related with the levels of serum Immunoglobulins and complements.

MATERIALS AND METHODS

i- Sample collection

Forty sera from patients with visceral leishmaniasis were collected, the age of those patients ranged from (1-9) year. And those patients diagnosed by testing the bone marrow in order to diagnose the infection, which is done in general Basrah hospital. Ten apparently healthy children were chosen as a control group.

Sera of patients and control group were tested by single radial immunodiffusion (SRID) to determine the concentration of IgG, IgM, IgA, C3 and C4.

ii- Single radial immunodiffusion (SRID)

SRID test was done by using Biomaghreb kit.

The plates were opened and left to stand at room temperature for a few minutes to allow any condensed water in the wells to evaporate. The wells were filled with 5μl of testing sera (patients and controls). Then left the plates to stay at room temperature for about (48) hours in the case of IgG, IgA, C3 and C4 and for (72) hours in the case of IgM.

The diameter of each immunoprecipitating ring formed around each well was measured in mm by using immunoviewer and the concentration of each immunoglobulin class and complement...
was calculated from standard curve in the kit test which represents the correlation between the diameter of ring in mm and concentration of antibody in mg/dl.

iii- Statistical analysis
The data analyzed by using analysis of variance (ANOVA) test.

RESULTS
Present results of (SRID) revealed that there were a highly significant differences (P<0.001) in mean levels of serum IgG and IgM between patients (1488.5, 168.9) and control group (617.9, 119.83) respectively, tables and figures (1, 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Range</th>
<th>Mean</th>
<th>SD±</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>40</td>
<td>981.3 - 1946.2</td>
<td>1488.5</td>
<td>278.5</td>
<td>44.06</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>529.0 - 836.7</td>
<td>671.9</td>
<td>128.1</td>
<td>40.53</td>
</tr>
</tbody>
</table>

level of serum IgG in patients and control group.

Where:
No. = Number
SD = Standard deviation
SE = Standard error

Figure (1): Precipitin ring of serum IgG in tested sera

Table (2): Mean level of serum IgM in patients and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Range</th>
<th>Mean</th>
<th>SD±</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>40</td>
<td>81.5 - 227.1</td>
<td>168.9</td>
<td>151.04</td>
<td>14.30</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>76.3 - 141.4</td>
<td>119.83</td>
<td>141.74</td>
<td>40.51</td>
</tr>
</tbody>
</table>
Figure (2): Precipitin ring of serum IgM in tested sera.
Whereas IgA mean level didn’t show any significant differences (P> 0.05) between patients (191.73) and control group (225.62), table and figure (3).

Table (3): Mean level of serum IgA in patients and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>40</td>
<td>43.7 - 225.4</td>
<td>251.73</td>
<td>90.39</td>
<td>14.30</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>81.7 - 255.4</td>
<td>225.62</td>
<td>128.02</td>
<td>40.81</td>
</tr>
</tbody>
</table>

Figure (3): Precipitin ring of serum IgA in tested sera.
In the case of serum complement levels, present data represent a highly significant differences (P<0.001) in mean concentration of C3 and C4 between patients (170.31, 46.09) and control group (141.10, 27.40) respectively, tables and figures (4, 5).

Table (4): Mean level of serum C3 in patients and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Range</th>
<th>Mean</th>
<th>SD±</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>40</td>
<td>93.4 – 252.6</td>
<td>170.31</td>
<td>44.63</td>
<td>7.06</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>93.4 – 132.1</td>
<td>141.10</td>
<td>13.52</td>
<td>4.27</td>
</tr>
</tbody>
</table>

Figure (4): Precipitin ring of serum C3 component in tested sera.

Table (5): Mean level of serum C4 in patients and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Range</th>
<th>Mean</th>
<th>SD±</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>40</td>
<td>29.3 – 69.4</td>
<td>46.09</td>
<td>12.04</td>
<td>1.90</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>24.5 – 43.9</td>
<td>27.40</td>
<td>5.96</td>
<td>1.88</td>
</tr>
</tbody>
</table>
DISCUSSION

Leishmaniasis exist as a complex of diseases that from an immunological point of view, fall into two categories, the self-healing dermal forms such as Leishmania tropica and effectively non-healing visceral forms caused by L. donovani (5).

The humoral part of immunity which studied here demonstrated that the serum of person infected with visceral leishmaniasis have a high level of IgG and IgM in comparison with control group, and this may be indicated the role of these antibodies during the course of infection, the same results reported also by (6) when showed that the hamster produce IgG which directed against certain parasite surface membrane antigens, (7) also recorded high level of globulin mainly IgG, during visceral leishmaniasis. In addition, recent results were in agreement with the results of (8) and (9) whom showed that the patients with symptomatic kala-azar have a high titer of anti-leishmanial antibodies. (10) showed that the promastigote of L. donovani was affected by normal human serum in vitro. Moreover, the importance of IgG, IgM and natural antibodies was also suggested by (1) and (11). Massive hypergammaglobulinemia was also documented by (12).

SRID results was not indicated any differences in serum concentration of IgA between patients and control group, and this may be due to that the infection trigger the classical complement pathway which activate initially by IgG and IgM not by IgA (13). So there was an increasing in the concentration of IgG and IgM but not IgA. Or may be due to that the L. donovani antigens do not induce the production of IgA.

The increasing in the concentration, of C3 and C4 may be contribute to that the infection appeared to activate the complement cascade through the classical pathway which is needed for expression of the kollal effect (1). (13) also noted the same observations that after inoculation the promastigote interacts with opsonic serum factors and activates the complement system, the third component of complement (C3)-coated parasite adheres to mononuclear phagocytes through CR1 and CR3 complement receptors.
Finally, the considerable humoral immune response was triggered during visceral leishmaniasis infection, which documented during this study revealed the cooperation between IgG, IgM, C3 and C4 in defending mechanism against the infection via the opsonization and then phagocytosis.

REFERENCES