Relevance of IL-4, IL-5 and IL-6 in Women with Toxoplasmosis in Erbil.

Sawsan M. Al-Sorchee, Nasir I. Khatab, Cihan University, College of Science, Biology Department, Salah-Aldeen University, College of Education, Biology Department, email:Sawsan_Sorchee@yahoo.com

Abstract

**Background:** Toxoplasmosis is a common infection in women with child bearing age and may attribute to bad obstetric outcomes.

**Aim:** This study was conducted to evaluate IL-4, IL-5 and IL-6 levels in women with toxoplasmosis and their relevance to this infection.

**Materials and Methods:** Sixty eight women were included in this study with a history of abortion (single or repeated). They were referred to by physicians from different hospitals and private laboratories in Erbil/ Kurdistan region of Iraq from March 2010 until March 2011 for the detection of anti-toxoplasma antibodies and 30 healthy women were selected as control. Venous blood samples were collected from these women; serum was obtained for ELISA test for the detection of anti-toxoplasma IgG and IgM. Levels of IL-4, IL-5 and IL-6 in these women were also detected.

**Results:** Fifty one women (75%) who had a single or repeated abortion were infected with Toxoplasmosis and had positive ELISA tests. From those, eleven (21.6%) had IgM, twenty five (49%) had IgG and fifteen (29.4%) had both IgM and IgG. The levels of IL-4, IL-5 and IL-6 were significantly high compared to the control.

**Conclusion:** Cellular immune response was highly involved during acute and chronic Toxoplasmosis.

Introduction

Toxoplasmosis is an infection caused by the intracellular parasite *Toxoplasma gondii*, which can be found in homoeothermic animals (including humans). Human infections occur due to the ingestion of oocysts or infective tissues which are present in raw or undercooked meat, contaminated water or soil [1]. Infections are generally benign in healthy adults. However toxoplasmosis is one of the major causes of death among immune deficient people such as AIDS patients. It can be transmitted to the fetus in uterus through transplacental transmission [2]. Primary infection during pregnancy can result in abortion or fetal defects [3].

Toxoplasmosis stimulates both the humoral and cell-mediated immunity which are essential for host control of intracellular infections [4]. It can stimulate the humoral immune response leading to antibody production including IgM and IgG antibodies. The presence of these two antibodies in serum is sufficient to define the individual as being acutely infected, while the presence of IgG only in the serum sample is sufficient to define this sample as being from chronically or past infected patient [5].

Toxoplasmosis stimulates the cell-mediated immunity (CMI) as well. This occurs when the *T. gondii* parasite rapidly promotes the production of
interleukin-12 (IL-12) most likely from dendrites cell. The production of IL-12 will then activate and trigger the natural killer and T- cells to synthesize interferon – γ (IFN-γ) and Interleukin - 4 (IL-4) [6, 7]. Thus the acute phase of toxoplasmosis can be distinguished by the increased levels of IFN-γ, IL-4 and IL-12 as well as other inflammatory cytokines such as TNF-α, IL-6 and IL-1 in the serum samples. IFN-γ has an important role in controlling resistance to acute [8] and chronic infection by *T. gondii* [9]. Al-Fertosi et al [10] showed that both IFN-γ and IFN-γ R1 percentage in women with toxoplasmosis were higher when compared with the negative group.

Salman et al [11] found that the level of apoptotic cells was higher in *T. gondii* infected cells than the uninfected cells. Apoptosis results from the over production of inflammatory cytokines. IL-4 has down-regulatory effects on IFN-γ [12, 13]. This study was conducted to evaluate IL-4, IL-5 and IL-6 levels in women with toxoplasmosis and their relevance to this infection.

**Materials and Methods**

Sixty eight women who had abortion were selected for this study. They were referred to different hospitals and private laboratories around Erbil city / Kurdistan region of Iraq, indicating the possibility of having toxoplasmosis by the physician. Venous blood was collected from those women for serum collection during the period between March 2010 and March 2011. The serum samples were divided into three groups according to the presence or absence of specific anti- *Toxoplasma* antibodies: The first group included 25 serum samples containing IgG, another group included 11 serum samples containing IgM and the third group included 15 serum samples containing IgM and IgG giving a total of 51. The other 17 patients were excluded from this study due to the absence of specific anti *Toxoplasma* antibodies in their sera.

Thirty healthy looking age– matched women were selected as controls. Venous blood was collected for serum collection which was tested for anti – *Toxoplasma* antibodies. Five of these revealed the presence of IgG and so were excluded from this study leaving a total of twenty five controls. For all tests performed, the procedures were repeated twice for each sample and the mean of the results were recorded.

This test was performed by the use of two kits (Omega Diagnostics Company, Scotland), one for the detection of IgG antibodies against *T. gondii* antigens in the patients’ serum, and the other for the detection of IgM antibodies against *T. gondii* antigens in the patients’ serum. Serum levels of IL-4 IL-5 and IL-6 were measured by the means of enzyme immunoassay using ELISA kits (Mabtech AB, Sweden). The study protocol was approved by the ethical committee of college of Education, Salah Aldean University and verbal informed consent was taken from each subject before her enrollment in the study.

**Statistical Analysis:**

Statistical analysis included calculation of the mean ± standard error (SE), the confidence interval that puts a lower and upper limit to the mean and considered upper 99% confidence limit. The t-test was adopted to check for any significance of the difference between infected and controls. The correlation coefficient (r) was calculated to reflect an association between parameters as a quantitative description to the relation. All statistical analyses were carried out as described before (14).

**Results**

The results showed that of the 68 women who had abortion (single or
repeated), only 51 (75%) were positive for toxoplasmosis and had antibodies of IgM and IgG by using ELISA (Table 1). The rest (17) of the women with abortion out of 68 (25%) were negative for the ELISA test and so were executed from the study. The serum samples of the 30 healthy women were tested for IgG and IgM specific antibodies for T.gondii by using ELISA in order to find out whether they were negative or positive before using them as control in the study. The results showed that not all of them were negative, 5 out of 30 of the healthy women (16.7%) were positive for either IgG or IgM antibodies and so were excluded from the study and only 25 healthy women were used as controls.

Table 1: Anti- Toxoplasma ELISA tests on the serum samples of women with abortion and healthy women.

<table>
<thead>
<tr>
<th>Case</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women with abortion</td>
<td>51</td>
<td>17</td>
<td>68</td>
</tr>
<tr>
<td>Healthy Women</td>
<td>5</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>42</td>
<td>98</td>
</tr>
</tbody>
</table>

The mean serum levels of IL-4 were significantly (P<0.0001) higher in the three groups as compared to controls and the difference was more than two fold, Table 2. However, there were no significant differences between the three groups. IL-5 mean serum levels were significantly in the three groups as compared to controls, but no significant difference between the three groups, Table 2. IL-6 mean serum levels were significantly higher in all the 3 groups than that in controls, but less than that for IL-4 and IL-5.

Table 2. The mean levels of IL-4, IL-5 and IL-6 in cases with positive toxoplasma.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean [SE]</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-4</td>
<td>IL-5</td>
<td>IL-6</td>
</tr>
<tr>
<td>Patients with IgM</td>
<td>11.81 [0.44]</td>
<td>6.46 [0.35]</td>
<td>5.54 [0.35]</td>
</tr>
<tr>
<td>Patients with IgG</td>
<td>11.56 [0.29]</td>
<td>6.04 [0.88]</td>
<td>4.68 [0.88]</td>
</tr>
<tr>
<td>Patients with IgM &amp; IgG</td>
<td>11.53 [0.30]</td>
<td>6.35 [1.02]</td>
<td>5.00 [1.02]</td>
</tr>
<tr>
<td>Control</td>
<td>5.25 [0.24]</td>
<td>2.15 [0.22]</td>
<td>2.50 [0.22]</td>
</tr>
</tbody>
</table>

Table 3 shows that the correlation coefficient (r- value) between IL-4 and IgM was found to be positive and highly significant (p≤ 0.01, r = 0.83), the same was for the coefficient between IL-4 and IgG (r = 0.71). The correlation coefficients between IL-4 and IgM and between IL-4 and IgG in the third group were positive but different (r = 0.59) and (r = 0.13) respectively. The correlation coefficient (r- value) between IL-5 and IgM was found to be positive (r = 0.22), while the coefficient with IgG was found to be negative (r
The correlation coefficients between IL-5 and IgM and between IL-5 and IgG in the third group were positive ($r = 0.34$) and ($r = 0.21$) respectively. The correlation coefficient ($r$- value) between IL-6 and IgM was found to be negative ($r = -0.17$), the same was for the coefficient with IgG ($r = -0.09$). The correlation coefficients between IL-6 and IgM and between IL-6 and IgG in the third group were positive ($r = 0.12$) and ($r = 0.25$) respectively.

Table 3: The correlation coefficient ($r$ value) of IL-4, IL-5 and IL-6 with antibody titer in women infected with T. gondii and with history of abortion.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive IgM</th>
<th>Positive IgG</th>
<th>Positive IgM &amp; IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>IgM</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.83</td>
<td>0.71</td>
<td>0</td>
</tr>
<tr>
<td>IL-5</td>
<td>0.22</td>
<td>-0.09</td>
<td>0</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.17</td>
<td>-0.09</td>
<td>0</td>
</tr>
</tbody>
</table>

**Discussion**

Toxoplasmosis is a worldwide prevalent disease [15]. Infection with T. gondii is common among humans, and it is estimated that one third of the world's population has been exposed [16,17]. Primary infection during pregnancy can result in abortion or fetal defects [3]. *Toxoplasma* infection in the human population varies geographically with prevalence rates approaching 90% in some European countries, while rates in the United States are between 10% and 15% [18, 19]. Additionally, the rate in Egypt is 65% [15], France is 71%, Ibadan and Nigeria is 87% [20].

In this study the rate was also high (75%) in women with single or repeated abortion who are seropositive. The percentage of women with past or chronic toxoplasmosis (with IgG only) was high (49%), while the percentage of women with acute toxoplasmosis (with both IgG and IgM) was (29.4%) and with IgM only was (21.6%). Specific IgM antibodies were with wide variation in reported and form 55.5% Iraq women [15]. The high prevalence of this disease in Iraq could be due to the high number of risk factors and many sources of infection. These include the ingestion of sporulated oocysts in soil (e.g. during gardening), eating under cooked meat contaminated with cysts, eating unwashed raw vegetables or unpadded fruits [16]. Weaker associations were observed for tasting raw meat during preparation of meals, eating salami, drinking unpasteurized milk and animal contact [21-23]. Serological surveys found the highest prevalence of Toxoplasmosis specific antibodies in rabbits 22.2% [24], 33% in dogs [25] and 70.6% in cats were sero positive [26]. Infection with *T. gondii* alters the specific *H. felis* immune response, converting a previously resistant host to a susceptible phenotype. Gastric...
mucosal IFN-γ and IL-12 were significantly elevated and IL-10 substantially reduced in dual-infected mice [27] also T. gondii induces lethal inflammatory cytokine shock in D-Gal-sensitized animals [28].

Toxoplasma-infected macrophages induce the production of IFN-γ, IL-2, IL-4 and IL-10 by CD4 (+) T-cells and that of IFN-γ and IL-2 by CD8 (+) T-cells. The production of IL-4 and IL-10 by CD4 (+) T-cells can suppress the IFN-γ-mediated mechanisms that protect the host against the parasite [29] thereby preventing host immunopathology [30,31]. Endogenous IL-4 is required for the induction of CD4+ Th1 protective antifungal responses [32]. CD4 (+) TL are divided into T helper 1 (Th1) and 2 (Th2). Mast cells interact with T. gondii and are massively infected, especially after their maturation by c-kit ligand [33].

Robert et al [12] found that the reduction in mortality during the early acute phases of infection may be due to the down-regulatory effects of IL-4 or associated Th2-derived products on proinflammatory cytokines such as IFN-γ. Th2 cytokines, such as IL-4 have been shown to be associated with progression of disease [12,34]. IL-4 plays a regulatory function during the innate immune response [35]. Allen et al [36] found that IL-4 produced in vivo in response to filarial L3 and adult parasites is essential for the induction of proliferative suppression but is not itself the suppressive factor [36].

IL-4 Knockout mice are more susceptible to acute T. gondii infection than are wild-type animals [10], also Th2 cytokine responses (IL-5, IL-10) were down-regulated in the IL-4 Knockout mice infected with Cryptococcus neoformans isolates [37]. Denkers et al [38] showed a protective role of IL-5 against T. gondii infection and suggest that IL-5 may play a role in the production of IL-12. Kijlstra et al [39] revealed the frequent presence of IL-6, IL-10, and IFN-γ in acute retinal necrosis (ARN) and toxoplasma chorioretinitis, whereas IL-2 and IL-4 levels remained below the detection limit.

In addition, the absence of this mediator may be beneficial to the host in surviving acute infection [40]. IL-4 is the main promoter of type-2 responses and is classically reported as counter-regulating type-1 immunity [41,42]. Johnson et al [43] found an important role for parasite-specific IgM in limiting systemic dissemination of tachyzoites during early acute T. gondii infection. This may indicate that IL-4 producing CD4 (+) cells contribute significantly to protection against infection with highly virulent as helper cells for production of isotype – switched antibodies [44].

The present study showed a significant increased level (more than two folds) of IL-4 in women infected with T. gondii (who had abortion) who had IgM, IgG and both IgM and IgG when compared with healthy control women. There were no significant differences among the groups. The correlation coefficient between IgM and IL-4 and also between IgG and IL-4 were positive and highly significant (r=0.83) and (r=0.71) respectively. Also IL-5 levels were increased but less significant than those for IL-4 compared with healthy control women. The correlation coefficient between IgM and IL-5 was positive (r=0.22)
but between IgG and IL-5 were negative (r = -0.09). IL-6 levels were increased but less significant than those for IL-4 and IL-5 compared with healthy control women. The correlation coefficient between IgM and IL-6 and between IgG and IL-6 were negative and significant (r = -0.17) and (r = -0.09) respectively.

In conclusion, this study suggests that the levels of IL-4, IL-5 and to a lesser extent IL-6 in acute and chronic Toxoplasmosis were not different which also suggests that IL-4 and IL-5 are associated with the progression of the disease.

References

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