THE REGULATION OF IMMUNE SYSTEM BY ANTI-INFLAMMATORY INTERLEUKINS IN HEART DISEASE PATIENTS INFECTED WITH TOXOPLASMOSIS

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ABSTRACT

The infection with Toxoplasmosis in humans, especially in people with weak immune system, pregnant women and those with underlying disease could entail serious damage. The objectives of this study are to calculate the prevalence ratio of Toxoplasma gondii infection in heart disease patients and assess important role of interleukin-10 in immune regulation. About 150 blood samples were collected from heart disease patients in Thi-Qar province and about 100 blood samples were collected from apparently healthy controls. Enzyme linked immunosorbant assay Test (ELISA) was used to test sera of patients and controls to detect anti-Toxoplasma Abs (IgG&IgM). While the positive samples with toxoplasmosis were tested to detect the levels of IL-10 and IL-12. The overall estimated seroprevalence was 46% in heart disease patients are higher than those in apparently healthy controls 20%, and there was a significant difference between them. The results of interleukins elucidated high levels of IL-12 82.85 pg/ml in heart disease patients infected with toxoplasmosis when compared with control  60.97 pg/ml, and there was significant difference between them. While there was no significant difference in IL-10 in both patients and controls. Also found a significant difference between female and male in patients and controls, in male was higher than those in female. The increased level of IL-12 in patients and controls with toxoplasmosis indicate to the activity of immune system in those patients.

Keywords: Interleukin, Toxoplasmosis, Heart disease, ELISA

1. INTRODUCTION:

Toxoplasmosis ranks high on the list of diseases that lead to the death of patients with AIDS. However, following the widespread usage of highly active anti-retroviral therapy (HAART) opportunistic infections including T. gondii in AIDS patients have been declining significantly (Dubey, 2010). Protection against T. gondii acquired via oral ingestion of tissue cyst provided by numerous non immunological
factors, including barriers that are both chemical and physical (Buzoni-Gatel and Kasper, 2007). Cytokines are protein messengers that convey information between and within the immune system via specific cell surface receptor molecules. Traditionally, Cytokines can be divided into pro-inflammatory cytokines and anti-inflammatory cytokines, based on global effects in animal models or direct effects on individual immune-cell populations (Stegall, 2010). Interleukins are small protein molecules that signal specific cells to regulate the immune systems of organisms, they are primarily synthesized by T cells, monocytes, macrophages and endothelial cells (Gomes, 2016). IL-12 is clearly important in initiating a strong and effective cell-mediated immunity against T. gondii tachyzoite, IL-10 appears to modulate both IL-12 and IFN-γ synthesis in vivo, avoiding an excessive immune response that could cause extensive inflammation and host tissue damage. IL-10 and IL-12 are two major antagonists involved in regulating IFN-γ synthesis during the initial phases of infection. (Butcher et al., 2005).

IL-10 is considered to be an inhibitor of Th1, Th2 and Th17 immune responses (O’Garra, 2007). IL-10 is a protein that also inhibits the synthesis of a number of cytokines, including IFN-γ, IL-2, IL-3, of IL-12, IL-6, , miicroglia in the brain, and Granulocytes Macrophage - Stimulating Factor. In structure, IL-10 is a cytokine with anti-inflammatory properties including counteracting the function of Th1 lymphocytes. IL-10 plays a vital role in controlling the inflammatory response during chronic phase of T. gondii infection (Wilson et al., 2005). Presumed to keep homeostatic network and protect tissue from collateral damage caused by excessive inflammation (Owen et al., 2013).

The recent interest in IL-10 as a potentially protective cytokine in the atherosclerotic process is based on the hypothesis that the development of plaque-forming atherosclerosis is a localized inflammatory process of the vascular wall (Ross, 1999). Indeed, IL-10 may act as an antiatherogenic factor by a number of different mechanisms apart from mere inhibition of pro-inflammatory cytokines. First, IL-10 interferes with the first step in atherogenesis by inhibiting attachment of circulating immune cells to the endothelium through down-regulation of the adhesion molecules CD18, CD60L, and intercellular adhesion molecule-1 (ICAM-1) (Song et al., 1997). Second, IL-10 inhibits the secretion of chemotactic proteins by macrophages that might attract further leukocytes to the location of subendothelial inflammation. (Olszyna et al., 2000). Moreover, IL-10 reduces the production of lytic enzymes produced by monocytes, such as matrix-metalloproteinases, and suppresses superoxide anion production, suggesting that IL-10 could prevent plaque destabilization (Lacraz et al., 1995).

2. METHODS:

2.1 Sample Collection:

About 150 blood samples had been collected from patients with heart disease, in Thi-qar province. According to gender, the patients divide into (81 male and 69 female) with age ranging 18 and 82 years. Also, 100 blood samples had been collected from apparently healthy persons as negative control, dividing according to gender into (55 males and 45 females) with age ranging 19-56. Six millilitre of blood were collected from each person by using disposable syringes, and then the blood was placed in gel tubes and allowed to clot at room temperature, then centrifuged at 3000 round per minute (rpm) for 10 minutes and sera were dispensed into 4 eppendorf-tubes, and stored at -20 °C until further analysis.

2.2 Detection of anti-Toxoplasma gondii antibodies:

The Foresight Toxoplasma IgG and IgM ELISA (Foresight, USA) kit was used to evaluate the titer of Abs against Toxoplasmosis. This assay was performed according to manufacturer’s procedure.

2.3 Calculate of serum IL-10 and IL-12 levels by ELISA technique:

The Elabscience Interleukin (IL-10 and IL-12) ELISA kit was used to evaluate the titer of total Interleukin (IL-10 and IL-12) concentration in human sera by micro plate Enzyme Immunoassay.
This assay was performed according to manufacturer’s procedure.

2.4 Statistical analysis
SPSS version 20 was used to analyse the data in present study with chi-square, P value < 0.05 and T test were considered statistically significant.

3. RESULTS:

The sero-prevalence ratio of anti-Toxoplasma Abs (IgG and IgM) in both subjects was 69 of 150 (46%) in heart disease patients, while in controls were 20 of 100 (20%), the current results indicate there is significant differences between them at p ≤ 0.05, as summarised in table (1).

Table 1: Seroprevalence of toxoplasmosis in study groups

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>ELISA test</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anti-Toxoplasma positive</td>
<td>Anti-Toxoplasma negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>Heart disease patients(150)</td>
<td></td>
<td>69</td>
<td>46</td>
<td>81</td>
</tr>
<tr>
<td>Control (100)</td>
<td></td>
<td>20</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>89</td>
<td>35.6</td>
<td>161</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td></td>
<td>X²=17.691, Df=1, P≤0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results show existence of anti-Toxoplasma IgG Abs in both sex, and the higher value in male of heart disease patients was 44 of 81 (54.3%), also the higher value of anti-Toxoplasma IgG Abs in male of controls was 14 of 55 (25.4%), whereas the lower value in female in heart disease patients was 25 of 69 (36.2%), and the lower value of anti-Toxoplasma IgG Abs in apparently healthy was showed in female 4 of 45 (8.9%). The results indicate to there is a significant differences at (p ≤ 0.05) between male and female in the seropositivity of anti-Toxoplasma Abs in both subjects, while no significant difference between both gender in the IgM results at (p ≤ 0.05), table (2).
Table 2: Existence of anti-Toxoplasma Abs in heart disease patients and apparently healthy controls distributed according to gender

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>gender group</th>
<th>ELISA test</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG+ve</td>
<td>IgG-ve</td>
<td>IgM+ve</td>
<td>IgM-ve</td>
<td></td>
</tr>
<tr>
<td>Heart disease patients</td>
<td>Male</td>
<td>44</td>
<td>54.3</td>
<td>37</td>
<td>45.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>25</td>
<td>36.2</td>
<td>44</td>
<td>63.8</td>
<td>1</td>
</tr>
<tr>
<td>Control (100)</td>
<td>Male</td>
<td>14</td>
<td>25.4</td>
<td>41</td>
<td>74.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>8.9</td>
<td>41</td>
<td>91.1</td>
<td>2</td>
</tr>
</tbody>
</table>

$X^2=4.908, \text{ Df}=1, P \leq 0.05$

$X^2=1.182, \text{ Df}=1, P > 0.05$

In the present study the level of IL-10 and IL-12 were calculated by ELISA in both group (heart disease patients and controls), and showed elevated in the level of IL-12 (82.85 pg/ml) in heart disease patients compared with controls was (60.97 pg/ml) and there was significant difference $p<0.05$ between them. On the other hand no significant difference between heart disease patients and controls in the results of IL-10 was (7.18 pg/ml) in heart disease patients compared with controls was (8.14 pg/ml), as shown in Table 3.

Table 3: The Interleukin levels in heart disease patients with toxoplasmosis and controls

<table>
<thead>
<tr>
<th>Interleukins</th>
<th>Groups</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL12</td>
<td>Patients (Anti-Toxoplasma positive)</td>
<td>82.85*</td>
<td>58.95</td>
</tr>
<tr>
<td></td>
<td>Controls (Anti-Toxoplasma negative)</td>
<td>60.97</td>
<td>17.23</td>
</tr>
<tr>
<td></td>
<td>$t=2.382$, significant P value =0.020</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*There is a significant difference between groups at $\alpha 0.05$

| IL10         | Patients (Anti-Toxoplasma positive) | 7.18  | 3.71           |
|              | Controls (Anti-Toxoplasma negative) | 8.14  | 2.30           |
|              | $t=-1.299$, significant P value =0.199 |       |                |

There is no significant difference between groups at $\alpha 0.05$

Also we calculated the level of interleukins in controls with Toxoplasmosis, and who was not have anti-Toxoplasma, and the results demonstrate there is significant difference at $(p<0.05)$ in the level of IL-12 between
controls with toxoplasmosis (IL-12 = 220.24 pg/ml), and control Anti-Toxoplasma negative (IL-12 = 60.97 pg/ml). While there was no significant difference in IL-10 between both groups, the level of IL-10 in Anti-Toxoplasma positive was (IL-10 = 9.14 pg/ml) and IL-10 in Anti-Toxoplasma negative was (8.14 pg/ml), as in table (4).

**Table 4:** The interleukin levels among control with *Toxoplasmosis* and who was not have anti-Toxoplasma Ab.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL12 Anti-Toxoplasma positive</td>
<td>220.24**</td>
<td>165.96</td>
</tr>
<tr>
<td>IL12 Anti-Toxoplasma negative</td>
<td>60.97</td>
<td>17.23</td>
</tr>
<tr>
<td>t= 4.052, significant P value =0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL10 Anti-Toxoplasma positive</td>
<td>9.14</td>
<td>2.74</td>
</tr>
<tr>
<td>IL10 Anti-Toxoplasma negative</td>
<td>8.14</td>
<td>2.30</td>
</tr>
<tr>
<td>t= 1.207, significant P value =0.236</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*There is a significant difference between groups at α 0.05, 0.01

There is no significant difference between groups at α 0.05

**DISCUSSION:**

In the study indicated that anti-Toxoplasma Abs (IgG) was (46%) in heart disease patients and (20%) of apparently healthy controls, the percentage of anti-toxoplasmosis Abs (IgG) in overall subjects was (32.8%), are higher than those during acute infection (IgM) was (1.2%), this was higher than results of anti-toxoplasmosis Ab(IgG) of other studies in the same city by Al-Ghezy, (2012) and Al-Mosawai, (2014) who indicated that the seroprevalenceIgG Toxoplasma Abs was 17.5% and 11.91% respectively, while the seroprevalenceIgM Toxoplasma in our study was lower than the seroprevalenceIgM in same city by Al-Ghezy, (2012) and Al-Mosawai, (2014) who indicated that the seroprevalenceIgM Toxoplasma antibodies was 3.4%and 5.33% respectively.

The result of current study was matched with other finding by Tallab et al.,(2012) who recorded that the rate of prevalence anti-Toxoplasma Abs in heart disease patients was 37.9%. The was corresponding with previous studies in other provinces in Iraq by Tawfeeq et al.,(2012) and Alkhanaket et al.,(2015) which recorded that the seroprevalenceIgG Toxoplasma antibodies was 32.4%and 35.31% respectively. The results in the present study could be explained by the detail that the group examined consisted of heart disease patients and apparently healthy persons, and IgG positive persons were infected with latent toxoplasmosis without a persistence of IgM antibodies after acute infection in the past(Carmen et al., 2006).

The presence of anti-Toxoplasma Abs in females was lower than those in males within both subjects, This finding is corresponding with other previous studies by Yassin, (2015) which found higher percentage of seropositivity in males (71.4%) than females (28.6%). In contrast other studies by Kheirabadi et al.,(2013) who found the seroprevalence of anti-Toxoplasma Abs in females higher than males. Whereas other previous studies showed no significant difference between males and females in seroprevalence of toxoplasmosis (Aboelhadid et al., 2013). This may be due to many factors including lower immunity to *T.gondii* males as compared to females, social activities and different occupational. Also Females are more resistant than
males to parasitic infection because of testosterone immunosuppressive properties and gender associated differences in exposure (Morales-Mortar et al., 2004). Levels of immunoglobulin’s including IgM, IgG in females are higher than those in males with toxoplasmosis (Al-Qureshi, 2004).

The results showed elevating in the level of IL-12 in heart disease patients and apparently healthy controls, whom infected with toxoplasmosis comparing with non-infected with toxoplasmosis, this results was matched with results of other studies by Alkhanak et al., (2015) who found increased in the level of IL-12 in persons with asymptomatic toxoplasmosis in comparison with free-toxoplasmosis persons. Also the results in present study was matched with other previous study by Gomes et al., (2014) who scored increased in the level of IL-12 in persons infected with Toxoplasma in skeletal muscle cells (SkMC), and the present study was different from others Al-Khafajhi et al., (2011) who found reduced in the level of IL-12 in women infected with toxoplasmosis in comparison with free-toxoplasmosis women.

Production of IL-12 and IFN-γ is essential to control infection by T. gondii. IFN-γ synergizes with IL-12 to drive the differentiation of Thp to Th1 phenotype, express IL-12 receptor on T cells, and inhibit the antagonist IL-4 to prevent the differentiation of Thp towards Th2 phenotype (Cordeiro et al., 2008). The role of IL-12 in toxoplasmosis including induces production of IFN-γ from natural killer (NK) cells and T cells, IFN-γ signaling promotes the development of a number of activator of transcription 1 (STAT1)-dependent anti-parasitic effector mechanisms, including reactive oxygen intermediates (ROI) production and p47 GTPaseupregulation (Tait and Hunter, 2009).

On the other hand, the results of IL-10 showed no significant difference between heart disease patients and controls and this may be because the increasing level of IL-12 that have antagonist effect with IL-10. This low level of IL-10 during chronic or past infection with toxoplasmosis in the current study matched the results of other studies (Al-Khafajhi et al., 2011 and Abdullah et al., 2011) who found reduced in the level of IL-10 in women infected with toxoplasmosis in comparison with free-toxoplasmosis women, IL-10 plays a vital role in controlling the inflammatory response during chronic phase of T. gondii infection (Wilson and Wille-Reece, 2005). IL-10 serves a dual important role during acute toxoplasmosis, by it inhibits IFN-γ production and the proliferation of T-lymphocytes, thus preventing a potentially protective Th1 immune response. Such T-cell-dependent immune suppression exerted by IL-10 primarily appears to avoid overwhelming inflammation which eventually leads to death. IL-10 may also deactivate macrophages, thus reducing IFN-γ-induced toxoplasmacidal activity and facilitating intracellular parasite survival. Hence, IL-10-induced immune suppression following infection with T. gondii is beneficial for both the parasite and the host and favors a stable host - parasite relationship (Ellis-Neyeret et al., 1997).

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