Study of Prevalence and Some Immunological Characteristics of *Toxoplasma gondii* Infections in Pregnant Women

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Abstract

In this study, 182 pregnant women suffering from miscarriage come to the Babylon Maternity and Children Hospital from October / 2013 to January / 2014. These women submit to a history and physical examination. Blood samples from pregnant women were tested for IgG and IgM. Antibodies and sociodemographic information were collected. Their average age was 28.1 years. The study showed that anti- *Toxoplasma* IgG, IgM, mixed IgG and IgM and overall seropositivity of all antibodies were 13.37%, 6.04%, 7.14% and 29.64% respectively.

There was significant effect of age on proportion rate. The highest infection rates were found at 26-30 age group, while the lowest infection rate found at 36-40 age groups. The total prevalence was more in third trimester of pregnancy (9.73%). IgG was more concentrated in third trimester (12.5%) than IgM (6.22%). No significant difference was found between residency and the prevalence of *Toxoplasma gondii* antibodies in both rural and urban areas.

**Keywords:** Toxoplasma gondii Infection, Toxoplasmosis

Introduction

*Toxoplasma gondii* that causes the disease toxoplasmosis is an obligate intracellular parasitic protozoan (Dardé et al, 2011). Serological studies evaluate that up to a third of the global population has been exposed to and may be chronically infected with *T. gondii*, although infection rates differ significantly among countries (Pappas et al, 2009).

Transmission of *toxoplasma gondii* occurs mostly by the ingestion of contaminated raw / undercooked meat with tissue cysts, as well as food borne way through the ingestion of contaminated vegetable / water with oocysts (Gajadhar et al, 2006). The European Food Safety Authority (EFSA) has documented toxoplasmosis as parasitic zoonosis with the highest human incidence. Researchers consider the eating of undercooked infected meat as the biggest risk and suspected ovine meat, to be a major risk factor for human infection (EFSA, 2007).

Enzyme-linked immunosorbent assay (ELISA), also known as an enzyme immunoassay (EIA), is a biochemical technique used generally in immunology to detect the presence of an antibody or an antigen in a sample. In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface of wells, and then a
specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme by use conjugated (enzyme-Ab), and in the final step a substance is added that the enzyme, and this can convert to some detectable signal, most commonly a color change in a chemical substrate. The detection of specific IgM & IgG antibodies has been used serological marker for diagnosing recent toxoplasmosis (Suzuki et al, 2001). The purpose of this study was to determine the incidence of Toxoplasmosis in pregnant women in Al-Hilla city from October 2013 to January 2014.

**Method**

In this study 182 blood sample of pregnant women suffering from miscarriages were collected from patients who come to the Babylon Maternity and Children Hospital from October/2013 to January /2014. These women submit to a history and physical examination to study occurrence of *T. gondii*. The age of patients ranged between (15- 40) years old. Five ml blood samples were collected by vein puncture from all studied women after sterilizing the skin with 70% alcohol. Blood samples were collected in plastic tubes and left to clot for about 30 min. at room temperature, then they were centrifuged for five minutes at 3000 rpm and separated sera were moved into other tubes (Voler and Bidwell, 1985). The collected sera were stored at -20C until used for the required test in the Central Public Health Laboratory/Babylon; where the ELISA test has been done. For determination of *T. gondii* antibodies (IgM and IgG), we used a third-generation of enzyme immunoassay kits for *T. gondii* IgM and IgG ELISA kits/ BioCheck-USA.

**Results**

From (182) miscarriage's women, 49 (26.92%) serum sample were positive for anti- *Toxoplasma* antibodies and this positive results were diagnosed as 11 (6.04%) for anti IgM antibody and 25 (13.73%) for anti IgG antibody whereas 13 (7.14%) samples were positive for both of anti IgM and anti IgG antibodies (figure-1).

![No.seropositive sample](image)

**Figure(1) Distribution of Toxoplasmosis (Anti IgM and IgG) by ELISA Test.**
Table(1): Seropositive of Toxoplasmosis According to number of Miscarriages by Using ELISA Test.

<table>
<thead>
<tr>
<th>Miscarriages NO.</th>
<th>Total Tested Number</th>
<th>Positive Samples Number</th>
<th>Seropositive Samples Types</th>
<th>IgM %</th>
<th>IgG %</th>
<th>IgM &amp; IgG (%)</th>
<th>(%) From Seropositive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>9</td>
<td>3</td>
<td>7.50</td>
<td>4</td>
<td>10.00</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>69</td>
<td>13</td>
<td>3</td>
<td>4.34</td>
<td>6</td>
<td>14.49</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>9</td>
<td>3</td>
<td>10.71</td>
<td>4</td>
<td>21.42</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>8</td>
<td>1</td>
<td>5.62</td>
<td>5</td>
<td>31.57</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>10</td>
<td>1</td>
<td>3.84</td>
<td>6</td>
<td>26.92</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>182</td>
<td>49</td>
<td>11</td>
<td>6.04</td>
<td>25</td>
<td>13.37</td>
<td>13</td>
</tr>
</tbody>
</table>

\[X^2(\text{cal})=19.593\] \[X(\text{tab.})=3.205\] (P-value. <0.05)

Table (1) exposed the relation between number of miscarriage and seropositive samples which involved in this study. The highest percentage of IgM antibodies incidence was in women suffering from 3 miscarriage times (10.71%) with a significant difference comparing with other groups of miscarriage times in the studied women (P-value. <0.05). While the highest percentage of IgG antibodies seropositive was in women with 4 miscarriage times (31.57%) with a significant difference comparing with other groups of miscarriage times in the studied women (P-value. <0.05).

Table (2) Anti Toxoplasma Antibodies and Percentage in Miscarriages Women According to The Age Groups by ELISA Test

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Control Groups (Negative)</th>
<th>Total Tested Samples</th>
<th>Seropositive</th>
<th>IgM %</th>
<th>IgG %</th>
<th>IgM &amp; IgG</th>
<th>% From Seropositive</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-20</td>
<td>4</td>
<td>32</td>
<td>2</td>
<td>6.25</td>
<td>2</td>
<td>6.25</td>
<td>2</td>
</tr>
<tr>
<td>21-25</td>
<td>8</td>
<td>41</td>
<td>2</td>
<td>4.87</td>
<td>4</td>
<td>9.75</td>
<td>3</td>
</tr>
<tr>
<td>26-30</td>
<td>7</td>
<td>52</td>
<td>4</td>
<td>7.69</td>
<td>12</td>
<td>23.02</td>
<td>5</td>
</tr>
<tr>
<td>31-35</td>
<td>3</td>
<td>30</td>
<td>2</td>
<td>6.66</td>
<td>4</td>
<td>13.13</td>
<td>2</td>
</tr>
<tr>
<td>36-40</td>
<td>3</td>
<td>27</td>
<td>1</td>
<td>3.70</td>
<td>3</td>
<td>11.11</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>182</td>
<td>11</td>
<td>6.04</td>
<td>25</td>
<td>13.37</td>
<td>13</td>
</tr>
</tbody>
</table>

\[X^2(\text{cal})=15.604\] d.f.= 4 \[X(\text{tab.})=7.779\] (P-value≤0.05)
Table(2) showed IgG and IgM anti-toxoplasmosis percentage in patients distributed over different age groups; the highest percentage of acute infected patients was (7.69%) in the age group (26-30) years old, while the lowest percent (3.07%) was in the age group (36-40) years old while the highest occurrence of chronic infection were (23.02%) at the age groups (26-30) years.

Table (3): Distribution of Positive Samples for Toxoplasma According to Trimesters of Pregnancy

<table>
<thead>
<tr>
<th>Trimesters of Pregnancy</th>
<th>Total No.</th>
<th>Seropositive Samples</th>
<th></th>
<th></th>
<th></th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgM</td>
<td>%</td>
<td>IgG</td>
<td>%</td>
<td>IgM &amp; IgG</td>
</tr>
<tr>
<td>First Trimester</td>
<td>65</td>
<td>4</td>
<td>6.15</td>
<td>8</td>
<td>12.30</td>
<td>5</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>85</td>
<td>5</td>
<td>5.88</td>
<td>13</td>
<td>15.29</td>
<td>5</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>32</td>
<td>2</td>
<td>6.25</td>
<td>4</td>
<td>12.50</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>182</strong></td>
<td><strong>11</strong></td>
<td><strong>6.04</strong></td>
<td><strong>25</strong></td>
<td><strong>13.37</strong></td>
<td><strong>13</strong></td>
</tr>
</tbody>
</table>

Trimesters of Pregnancy

\[ X^2 \text{ (cal)} = 0.423 \quad \text{d. f.} = 2 \quad \text{ } X^2 \text{(tab)} = 4.605 \]

Table (3) revealed IgG and IgM anti-toxoplasmosis percentage in patients distributed over three trimesters of pregnancy.

**Toxoplasmosis and Residency:**

The high ratio occurred in rural patients (8.08%) depending on IgM and (3.61%) in urban. on the other hand, results showed for IgG antibodies (16.16%) in rural, whereas they were (10.84%) in urban patients as revealed in table (4):
Table (4): Anti Toxoplasma Antibody and Percentage According to Residency by ELISA Test

<table>
<thead>
<tr>
<th>Residence</th>
<th>Total No.</th>
<th>Seropositive Samples</th>
<th></th>
<th></th>
<th></th>
<th>Association(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgM</td>
<td>%</td>
<td>IgG</td>
<td>%</td>
<td>IgM&amp; IgG</td>
</tr>
<tr>
<td>Rural</td>
<td>99</td>
<td>8</td>
<td>8.08</td>
<td>16</td>
<td>16.16</td>
<td>5</td>
</tr>
<tr>
<td>Urban</td>
<td>83</td>
<td>3</td>
<td>3.61</td>
<td>9</td>
<td>10.84</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>182</td>
<td>11</td>
<td>11.69%</td>
<td>25</td>
<td>27%</td>
<td>13</td>
</tr>
</tbody>
</table>

\*X^2 (cal) = 7.542  \quad \text{d. f. =2}  \quad (\text{tab}= 2.706)

Discussion

The prevalence of \textit{T.gondii} infection in the current study was (26.92\%) in Babylon province and the highest percentage of acute infected patients was (7.69\%) in the age group (26-30) years old, while the lowest percent (3.07\%) was in the age group (36-40) years old while the highest occurrence of chronic infection was (23.02\%) at the age groups (26-30) years. There were similarities and difference between the recorded results and many others from different location around Iraqi province. In AL-Mosul, Al-Khaaffaf (2001) reported 69.2\% seropositive for this disease in the same patients groups, while 49.85\% were positive by Al-Wattari in Duhok (2005), and 60.21\%, by Abbas (2002) and by Al-Sorchee (2005) 80.6\% in Baghdad .Mohamed (2008) in Babylon who reported 43\% positive result. Such differences were expected as the disease occurrence depended on the environmental conditions that which directly affects on the survival and spread of this parasite.

Other study likes Al-Rubiayi (2008) refers to the presence of more than one risk factor may influence the occurrence of toxoplasmosis as the sanitary conditions and habits of people. In Arab countries such as Egypt, El Deeb, \textit{et al.} (2012) has reported that (67.5\%) are seropositive for \textit{T.gondii} and in Saudi Arabia are (38\%) (Al-Almogren, 2012) and (30.8\%) are seropositive in Qatar (Marawan,2010). In other areas of the world, the present results are nearly similar to what reported in Nigeria, 46\% in Tanzania and 47\% in France (rural area) (João \textit{et al},2011). But they are higher than what in united kingdom, (22\%) and in South Korea, (43\%) as reported by Carl (2006). This high prevalence of the disease may be due to the high number of risk factors and many sources of infection which include the under cooked meat contaminated with cysts, eating unwashed vegetables or raw meat ingestion of sporulated oocyst in soil (e.g. during gardening eating). such finding may be due to
difference in the immunological status of women under study at the period of samples collection and this agrees with (Kang et al., 2000). The percent of association antibodies is 13 (7.14%) from the seropositive, while it is 15 (12%) by Al-Rubbaiy (2008). The study of Al-Fakahany (2002) in Egypt refers to the percent of IgM (27.3%) and (36.4%) for IgG antibody. While this study shows (6.04%) for anti IgM and (13.37%) for anti IgG antibodies. It has observed that significantly greater incidence of abortion occurred in patients with high antibody titer of IgM antibody. This result is supported by (Jones, 1996). The presence of anti- T. gondii (IgG) antibodies in the sample of serum is not sufficient to institute the fact that the patient has been infected (Li et al., 2000), while the absence of IgG early or before pregnancy allows identification of women at risk for acquiring the infection (Montoya and Liesenfeld, 2004). However, IgG class of antibodies is generally helpful in the diagnosis of acutely presenting illnesses because these antibodies take 1-9 weeks to develop (Jones et al., 2003) and continues for months or years. This study shows the highest percentage of acute infection (27.27%) which happens with patients who have three miscarriages table (1), whereas the lowest percent at women suffering from five miscarriages (12.15%), and the association infection appeared to be high in women of three miscarriages. These results are in contrast to the results of Othman (2004) in Kirkuk who has found the high percentage of seropositivity (55.17%) are among pregnant women with single abortions, moreover, the results of Al-Maqdisy (2000) in Ninewah has found that women with two abortions has higher percent of seropositivity, (34.14%). Conversely, Al-Hamdani and Mahdi (1996) in Basrah have found that the women with five or more abortions have the highest frequency rate of T. gondii antibodies. These results may be attributed to another causes rather than Toxoplasma in addition to the presence of previous infections with Toxoplasma detected by the presence of specific antibodies and they do not necessarily account for abortion.

In Most cases, women abortion occurs at the end of the first trimester and the beginning of the second trimester which is considered as a critical period of gestation where the fetus is not well established in the uterus (Lee et al., 2000). Studies suggest that when mothers infected in the first trimester, 14% of fetuses become infected; as compared to 33% in the second (Roizen et al., 1995) whereas there is an increase in the possibility rate of fetal infection with progress pregnancy trimester that may cause fetal death or severe malformation incompatible with fetal viability that is considered as one mechanism leading to abortion as mentioned by (Tabbara et al, 1999) who believed that the early transmission is associated with severe effects in stillbirth. The high percentage of infection is in the second trimester of pregnancy 15.15% and 39.39% respectively. No significant effect of trimesters on the frequencies of toxoplasmosis is appeared during the recent study.

In spite of the number of patients from rural areas is higher but it is a part of the total; rural women are positive for anti-Toxoplasma antibody and this result may be due to the fact that many women in rural regions live in low personal hygiene, poor sanitary, inadequate proper treatment and low educational level about risk factors, all that lead to transmission of T. gondii. The role of health education is considered by James and Daniel (2000) as a main factor in decreasing the occurrence of this infection. The urban areas in Babylon province show a mixture of different socioeconomic classes. So many women in city center live in poor sanitary and low socioeconomic state as or even less than many of rural areas included in the present study; this resulted in significant similarities in the number of infected aborted women.
This result is consistent with Ageel (2003) in Tikrit, who has found that the seropositivity is higher in rural areas (36.36%) than center ones (32.05%), Othman (2004) in Kirkuk who found that the seropositivity is higher among pregnant women from rural areas (50.0%) than those in center areas (33.5%). This may be attributed to the presence of indoor cats, or even the stray one in the rural areas more than in the city, which is considered as a final host for *T. gondii* and a main source for spreading the infectious oocyst in the soil, water, vegetables and fruits used for daily consuming. But it is not consistent with a study in Ninewah by Ahmed (1995), who has found a higher prevalence among the city resident (38%) than the rural one (29%), also in Osorno (Chili) by Zamaorano et al. (1999) who has found a slight higher prevalence in the urban areas than the rural ones.

**Reference**


