SERODIAGNOSIS OF TOXOPLASMA GONDII IN HABITUALLY ABORTING WOMEN AND OTHER ADULTS FROM NORTH JORDAN

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Abstract. The prevalence of anti-Toxoplasma gondii antibodies in serum samples of 55 habitually aborting women, 46 women with normal pregnancies, 92 outpatient adults, and 180 University students from North Jordan was studied using the enzyme linked immunosorbent assay (ELISA). Sera from the habitually aborting group were also tested by the indirect immunofluorescent (IIF) test. No significant difference was found between the overall prevalence rates in University students, outpatient adults and women with normal pregnancies (29.3%, 22.4% and 28.1%, respectively). The prevalence in habitually aborting women exceeded three times that in women with normal pregnancies or in outpatient females (58.3%, 26.1% and 26.0%, respectively), and was approximately three times that in female University students (18.3%). The greatest difference in the prevalence rate between habitually aborting women and those with normal pregnancies or outpatient females was found in groups having the highest antibody level (≥ 100% of standard positive controls). A positive correlation between the results of the ELISA and those of the IIF test occurred at titres of ≥ 1:40 of the latter test in habitually aborting women.

Toxoplasma gondii is one of the most common parasites of man. Serological studies indicate that 20–90% of adult population have been infected with this parasite (Feldman and Sabin 1949, Feldman and Miller 1956). Infection in adults follows a transitory acute phase which is followed by a mild subclinical or asymptomatic chronic phase wherein latent cystic stages which localize mainly in the central nervous system and/or musculature may persist for several years without invoking any host tissue reaction (Tadros and Laarman 1982). However, the most serious manifestation of the disease occurs in congenital foetal involvement. In such cases, foetal toxoplasmosis progresses as a generalized systemic disease which is followed by the typical classical symptoms of choriorrhitinitis, hydrocephalus and cerebral calcification (Looke 1982). Abortion, miscarriage, stillbirth and postnatal death are the most well documented sequelae (Remington et al. 1961, Werner et al. 1963, Desmonts and Couveur 1974, Tadros and Laarman 1982, Looke 1982). Whether congenital toxoplasmosis could lead to habitual abortion or not is still a controversial issue. While Jansen et al. (1970) and Jacobs (1973) indicate that there is no conclusive evidence of a correlation between habitual abortion and T. gondii infection, Langer (1965), Garcia (1968) and Awan (1973) reported repeated congenital infections of offspring from the same mother. Indirect evidence that congenital toxoplasmosis could lead to frequent intrauterine foetal deaths comes from several serological studies (Jones et al. 1966, 1969, Hingorani et al. 1970, Sharif et al. 1973, Mahajan et al. 1976, 1981, Oumachigu et al. 1980, Kandi et al. 1979, 1980, Jozy and Zajac, 1983). In these studies, habitually aborting women always had a higher prevalence of anti-T. gondii antibodies than control non-aborting women.

In Jordan, habitual abortion is not uncommon. However, the role of toxoplasmosis in inducing abortion is still subject to investigation (Morsy et al. 1977). Recently,
Morsy and Michael (1980) and El-Khateeb (1980) surveyed the prevalence of anti-
*T. gondii* antibodies in Amman (Central Jordan). The prevalence of anti-
*T. gondii* antibodies in other parts of Jordan and the role of toxoplasmosis in habitual
abortion remain to be studied. In the present paper we report the use of the ELISA
technique to investigate the prevalence of anti-*T. gondii* antibodies in habitually
aborting women compared with other adult control groups from North Jordan.

**MATERIALS AND METHODS**

**Serum samples.** Blood samples were collected from 68 male and 82 female University students (age 18–22 years) attending Yarmouk University, Jordan. All the subjects were residents of Irbid, North Jordan, were apparently healthy and were not taking any medication. Other blood samples were collected from 28 males and 64 females (age 20–40 years) who were categorized as out-
patients visiting Jarash Government Hospital for various complaints. This group comprised
subjects living in Jarash (45 kilometres South of Irbid) and its suburbs. None of these subjects
were diagnosed clinically to have toxoplasmosis. Blood samples were also collected from 55
habitually aborting women (age 20–40 years) who had to date 3–7 abortions, and from 46 women
who had normal pregnancies and were of a matched age group. Some of the habitually aborting
women had babies early during their reproductive life. Abortion or fetal death occurred at
various periods post-conception in the habitually aborting group. In all of these groups, 4–5 ml of
blood was withdrawn by venipuncture, the serum was separated conventionally and stored at
−20 °C until further use.

**Sero-diagnosis.** The ELISA and IIF tests were used to sero-diagnose anti-
*T. gondii* IgG antibodies in the serum samples collected. For the ELISA, the 
*T. gondii* soluble antigen, standard positive control and negative control sera as well as the rabbit anti-human IgG conjugated with alkaline
phosphatase immuno enzyme (AKP) were all purchased from Dynatech AG Laboratories (Switzerland).
The substrate, p-nitrophenyl phosphate was purchased from Sigma Chemical Company (West
Germany). The ELISA technique was performed in 96 well microtitre plates as suggested by the
manufacturer of the kit (Dynatech AG Laboratories, Switzerland) and as described by Volter
et al. (1979a, b). Initially a chicken erythrocyte titeration was performed to determine the optimal anti-
gen system, positive control serum and AKP conjugated rabbit anti-human IgG dilutions which best
suit the assay. Dilutions of 1 : 80, 1 : 160 and 1 : 100 for the *T. gondii* antigen, the standard
positive and negative control sera as well as the AKP conjugated antibody, respectively, were
found to be optimal to carry out the ELISA. These dilutions as well as a dilution of 1 : 100 for
each test serum were used throughout the study. The test was performed in triplicate or quadrupli-
cates and each plate included wells with the following controls: standard positive serum, standard
negative serum, no Toxoplasma antigen, no AKP conjugated antibody and no substrate. At the end
of incubation the reaction was read at a wavelength of 450 nm using a Dynatech Autoreader.
The IIF was performed conventionally (El-Khateeb 1980) using fluorescein conjugated anti-
human immunoglobulin (Nordic Company, Holland) and Toxoplasma antigen (Bio Merieux,
France) which was diluted with phosphate buffered saline (PBS, pH 7.6) to give 10–30 organisms per
high power field.

**Statistics.** For the ELISA, the reactivity index (RI) for each test serum was calculated as follows:

\[ RI = \frac{OD_{positive \ control \ with \ Ag} - OD_{positive \ control \ without \ Ag}}{OD_{positive \ control \ with \ Ag}} \times 100 \]

where:

- OD = optical density at 450 nm
- Ag = *T. gondii* soluble antigen diluted 1 : 80 with 0.05M carbonate buffer at pH 9.6

Positive control = standard positive control serum diluted 1 : 100 in 1 % bovine serum albumin
(gammagene, Sigma Chemical Company in PBS).

The RI for standard negative control (1 : 100 dilution in 1 % bovine serum albumin in PBS)
was calculated in each plate. Test serum samples that had a RI of 50 % of standard positive
control serum or more were considered to be positive for anti-*T. gondii* antibodies. Such a level of
ELISA reactivity index to denote a positive assay was used by Yagore et al. (1983) in studies on the seroepidemiology of schistosomiasis japonica.

**RESULTS**

The results of serodiagnosis for anti-*T. gondii* antibodies in various adult groups from North Jordan are shown in Table 1. Among University students, the overall
prevalence (RI ≥ 50 %) in both sexes was 23.3 %. Males had a higher rate than females (33.8 % and 18.3 %, respectively). However, the percentage of females who had the highest RI (≥ 100 %) exceeded two times that of males (9.7 % and 4.4 %, respectively). Most males had a lower RI of 50–74 % (22.1 %). In outpatient subjects
living in Jarash, the overall prevalence of anti-*T. gondii* antibodies was 22.8 %. There was no significant difference between male and female groups for the various RI categories in samples from this group.

When the prevalence of anti-*T. gondii* antibodies was compared between habitually aborting women and women with normal pregnancies, a statistically significant
difference was observed (p > 0.005, chi square test). The prevalence rates in these
two groups were 56.2 % and 26.1 %, respectively (Table 1).

The greatest difference in prevalence between these two groups was noted in the highest RI category (≥ 100 %). The percentage of habitually aborting women which

| Table 1. ELISA reactive IgG antibodies to Toxoplasma gondii in various adult groups from North Jordan |

<table>
<thead>
<tr>
<th>Group</th>
<th>No. tested</th>
<th>Percent of subjects with following ELISA R. I.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50–74</td>
</tr>
<tr>
<td>University students</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>68</td>
<td>22.1</td>
</tr>
<tr>
<td>Females</td>
<td>82</td>
<td>4.9</td>
</tr>
<tr>
<td>Both</td>
<td>100</td>
<td>12.7</td>
</tr>
<tr>
<td>Outpatients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>28</td>
<td>7.2</td>
</tr>
<tr>
<td>Females</td>
<td>64</td>
<td>12.5</td>
</tr>
<tr>
<td>Both</td>
<td>92</td>
<td>10.9</td>
</tr>
<tr>
<td>Normally pregnant</td>
<td>46</td>
<td>13.1</td>
</tr>
<tr>
<td>Habitually aborting</td>
<td>55</td>
<td>26.1</td>
</tr>
</tbody>
</table>

* R. I. ELISA reactivity index computed as per Materials and Methods. A R. I. of 50 % or more of standard positive control serum was considered to be positive for anti-*T. gondii* antibodies.

**Table 2. Relationship of Toxoplasma gondii indirect immunofluorescent (IIF) test results to ELISA results in 55 habitually aborting women. ELISA results are grouped as positive (reactivity index ≥ 50 %) or negative (reactivity index < 50 %) of positive controls**

<table>
<thead>
<tr>
<th>IIF Titre (reciprocal)</th>
<th>No. of Sera in Group</th>
<th>ELISA Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>≤ 20</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>40</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>80</td>
<td>80</td>
<td>8</td>
</tr>
<tr>
<td>160</td>
<td>160</td>
<td>1</td>
</tr>
<tr>
<td>≥ 320</td>
<td>320</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
had such a high RI was three times that of women with normal pregnancies (12.6 % and 43.3 %, respectively). There was no significant difference in the prevalence rate between outpatients and women with normal pregnancies (25.0 % and 26.1 %, respectively).

The relationship between the results of the ELISA and IFF tests on serum samples from habitually aborting women is given in Table 2. Evidently, 73–90 % of the samples which showed a titre of 1 : 40 to 1 : 160 in the IIF were positive in the ELISA. All of the 4 samples which had a titre of 1 : 1280 in the IFF were also positive in the ELISA. In contrast, 30 % of samples which had a titre of 1 : 20 or less in the IIF were positive in the ELISA.

**DISCUSSION**

The present study demonstrates that the prevalence of anti-Toxoplasma antibodies in North Jordan is high enough to warrant careful consideration by public health workers and gynaecologists. Although the present investigation agrees with previous investigations carried out on toxoplasmosis in Central Jordan (El Khateeb 1980, Morsy and Michael 1980) variations do exist (Table 3). These variations may be attributed to different prevalence rates in various localities of Jordan and/or may reflect differences in the sensitivity of the diagnostic tests employed.

**Table 3. Anti-Toxoplasma antibodies in Jordanian adults**

<table>
<thead>
<tr>
<th>Locality</th>
<th>Sex</th>
<th>Percent Positive</th>
<th>Test</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amman University students</td>
<td>M</td>
<td>28</td>
<td>IIF</td>
<td>El Khateeb 1980</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>31</td>
<td>IIF</td>
<td>El Khateeb 1980</td>
</tr>
<tr>
<td></td>
<td>M + F</td>
<td>14</td>
<td>IFA</td>
<td>Morsy &amp; Michael 1980</td>
</tr>
<tr>
<td>Mentally retarded children</td>
<td>M + F</td>
<td>18.4</td>
<td>ID</td>
<td>Morsy &amp; Michael 1980</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>18.3</td>
<td>IFA</td>
<td>Morsy &amp; Michael 1980</td>
</tr>
<tr>
<td>Irbit University students</td>
<td>F</td>
<td>37.1</td>
<td>Dye Test</td>
<td>Morsy &amp; Michael 1980</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>33.8</td>
<td>ELISA</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>33.7</td>
<td>ELISA</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>M + F</td>
<td>18.3</td>
<td>ELISA</td>
<td>Present study</td>
</tr>
<tr>
<td>Jerash Non-aborting</td>
<td>M</td>
<td>29.1</td>
<td>ELISA</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>19.9</td>
<td>ELISA</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>M + F</td>
<td>22.0</td>
<td>ELISA</td>
<td>Present study</td>
</tr>
</tbody>
</table>

The prevalence rates of anti-Toxoplasma antibodies in Jordan fit within the framework of the seroepidemiology of toxoplasmosis in the Middle East which ranges from 16–30 % (Rifai and Nagaty 1969, Abou Daoud and Schwabe 1960, Matsion 1973, Shours et al. 1973).

An association between habitual abortion and high incidence of anti-Toxoplasma antibodies is established in this study. The prevalence rate in habitually aborting women was more than double that in women with normal pregnancies or outpatients (Table 1). This is consistent with observations made elsewhere (Hiragorni et al. 1970, Sharif et al. 1973, Mahajan et al. 1976, 1981, Awan 1978, Oumachigu et al. 1980, Jezyna and Zajac 1983). Hiragorni et al. (1970) concluded that
REFERENCES


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