Comparative study between toxoplasmonic immunohistochemical and serological studies in pregnant women

By


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Abstract

This study aimed to evaluate immunohistochemistry in diagnosis of toxoplasmosis in pregnant women by comparing it with ELISA test, histopathological examination and immunohistochemical examination. The study was carried out on 100 pregnant ladies aging from 16 to 44 years old attending the Obstetrics and Gynecology department of Benha University hospital, 75 patients had complicated pregnancies while, 25 cases with normal pregnancies were taken as a control group. Immunohistochemical examination of the D&C and placental samples of the studied groups revealed that, the highest percent of apoptosis was detected among aborted cases. The current study shows that, the total positive IgG among studied groups was 41% while the total positive IgM was 11%. The number of seropositive cases for Toxoplasma IgG by using ELISA was; 22 (59.4%) aborted females out of them 2 (5.4%) showed mild elevation, 18 (48.6%) moderate elevation and 2 (5.4%) marked elevation, no marked elevation in IUFD and PTL cases. The difference between groups were statistically insignificant (P>0.05). The number of seropositive cases for Toxoplasma IgM by using ELISA was 7 aborted females. Out of them, 2 cases showed mild elevation, 4 cases showed moderate elevation and one case showed marked elevation. While, 3 cases of PTL were seropositive. The difference was statistically insignificant (P>0.05). the histopathological examination of the D&C and placental samples of studied groups revealed that, the highest number of cases having villitis is 27 out of 37 aborted females. While, 2 females from control group have villitis ELISA IgM positive cases were 10 while cases had villitis by histopathological examination were 11 while by Immunohistochemical study they were 10. The control group showed one positive female by ELISA IgM.

Key words: Toxoplasmosis, immunohistochemical study, pregnancy.
Introduction

Infection with *Toxoplasma gondii* (*T. gondii*) is one of the most common parasitic infections of human and other warm blooded animals. It infects as much as 50% of the world's human population (*Nissapatorn et al., 2011*).

Villitis is an important placental lesion, which can be caused by specific maternal infections, e.g. rubella, toxoplasmosis, etc. The clinical significance of villitis depends on the etiology and severity of fetal infection and may lead to intrauterine fetal death, abortion, malformations and fetal growth retardation. The latter has been the major clinical association of villitis of the placenta (*Shiono et al., 2007*). Immunohistochemical method allows detection of *T. gondii* antigen and its apoptotic changes in formalin fixed specimens (*Silva et al., 2013*).

Aim of the work

The aim of this study was to evaluate Immunohistochemistry in diagnosis of toxoplasmosis in pregnant women by comparing it with ELISA test, Histopathological examination.

Subjects, Materials and Methods

**Ethics statement:** Before the study began, the study objective was explained to patients. Oral consent was taken. The study protocol was reviewed and approved by the ethical review board of the Faculty of Medicine. Benha University.

**Study type:** Cross sectional analytic study.

**Study place:** This study was conducted in the Obstetric and Gynecology Department of the Benha University Hospital.

**Study group:** One hundred pregnant ladies aging from 16 to 44 years old, 75 patients had complicated pregnancy while 25 cases with normal pregnancy were taken as control.

**Sampling:**
1) Blood samples were taken from all cases
2) Specimens of products of conception were sent for histopathological examination.
3) Conception products were sent for immunohistochemical examination.


(1) **Blood sample:** 10 ml of fresh blood were collected from one of the superficial veins using disposable plastic syringes in 2 separate 10 ml sterile tubes from selected patients and control. The first aliquote were used for routine investigation including:

- Rh group.
- *Treponema Pallidum* Haemagglutination test.
- Cytomegalovirus (CMV) IgM.
- Determination of Blood glucose level.

The second aliquote were centrifugated at 3000 rpm for 5 minutes, then the supernatant serum were collected in a clean sterile eppindorf tube for ELISA examination and the tubes containing sera samples were stored at – 40 C° till used.

**The following patients were excluded from the study :**

1- Patient had Rh negative factor  
2- Patient had positive *Treponema Pallidum* Haemagglutination test.  
3- Patient had positive IgM for Cytomegalovirus.  
4- Patients has recurrent complicated pregnancy.  
5- Patients received teratogenic medications.  
6- Patients with positive consanguinity.  
7- Patients who had heridofamilial problems.  
8- Diabetic patients.

*Every case of the study was subjected to the following:-*

**A - History taking:**

1- **Personal history:** Including name, age, address, occupation and had children or not.  
2- **Complaint:** On the patient own words e.g. vaginal bleeding.  
3- **Present history:** To analyze each complaint of the patient.  
4- **Menstrual history:** For detection of the first day of the last normal menstrual period to calculate the duration of pregnancy.  
5- **Obstetric history:**
    - Gravidity: number of previous pregnancies.  
    - Parity: number of previous deliveries.
- Complicated pregnancy either abortion, congenital anomaly, stillbirth, intrauterine fetal death, premature delivery and any other complication.

6- Past history:
- Of gynecologic or surgical operation.
- Of medical diseases such as T.B or DM.
- Past history of previous congenital anomalies or complicated pregnancy.
- Past history of medication that might be teratogenic as DES (diethyl stilbesterol).

7- Family history:
Of congenital anomalies or herido familial diseases.

B- Examination: It includes general, abdominal, chest and heart examinations to detect possible systemic diseases. Then local examination to confirm the pregnancy and possible duration.

C - Investigation
(1) Abdominal ultrasonography: It was done for all cases in the Obstetrics and Gynecology Department with an ultrasound scanner ATL 3500 and ALOKA 500 with a linear and convex probes. Its value was for.
   - Confirmation of pregnancy and its duration.
   - Number of pregnancy either single or multiple.
   - Either the fetus is living or dead.
   - Site of placenta.
   - Amount of liquor amnii.
   - Type of the present complication e.g. (abortion, congenital anomalies etc.).

(2) Laboratory investigation:
(a) Blood samples were examined by:
   Routine investigation for:
   - Rh.
   - TPHA.
   - CMV IgM
   - Blood glucose level. (Fasting and postprandial).
Then the Following was done:
- ELISA for *Toxoplasma* IgG.
- ELISA for *Toxoplasma* IgM.

(b) Histopathology of the products of conception.

(c) Immunohistochemical examination of the products of conceptions.

**Statistical analysis:** The statistical analysis was performed using statistical analysis system (SPSS); Version 16. Frequenct and percentage were used with qualitative data. Z test and Chi square were used to compare frequencies.

**Results:** The results was shown in table (1-3) and figure (1-4)

This current study shows that, the total positive IgG in studied groups was 41% the difference between the two groups was statistically insignificant (\(p>0.05\)), (table 1). The total positive IgM in studied groups was 11%. The difference between two groups was statistically insignificant (\(p>0.05\)), (table 2). The number of seropositive cases for *Toxoplasma* IgM by using ELISA was 7 aborted females. Out of them, 2 cases showed mild elevation, 4 cases showed moderate elevation and one case showed marked elevation. While, 3 cases of PTL were seropositive. The difference was statistically insignificant (\(P>0.05\)), (table 3)

This current study shows that, the number of females having positive IgG and negative IgM was 36/100, number of females has positive IgG and positive IgM was 5/100. Females have negative IgG and positive IgM was 6/100 and number of females has negative IgG and negative IgM was 53/100. *Toxoplasma gondii* positive females (cases and control) were 47 females (table 4). The number of positive cases by histopathological method in case group was 42 from them 40 were positive by ELISA IgG& IgM (table 5). While, 7 females from control group were positive by ELISA IgG& IgM and 2 out of them were positive by histopathological method, this was statistically significant (\(P< 0.001\)).

The present study shows that, the number of positive cases by ELISA IgG& IgM (table 6) and immunohistochemical method in case group was the same (40), while in control group 7 females were positive by ELISA IgG& IgM. Control group was negative by immunohistochemical method and this was statistically significant (\(P< 0.001\)).
Table (1): Results of serum IgG ELISA of the studied groups

<table>
<thead>
<tr>
<th>Serum IgG ELISA</th>
<th>Cases (no=75)</th>
<th>Control (no=25)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Positive</td>
<td>34</td>
<td>45.3</td>
<td>7</td>
</tr>
<tr>
<td>Negative</td>
<td>41</td>
<td>54.7</td>
<td>18</td>
</tr>
</tbody>
</table>

\[ X^2 = 1.7 \quad P => 0.05 (0.2) \]

Table (2): ELISA-IgM results in relation to studied groups:

<table>
<thead>
<tr>
<th>Serum IgM ELISA</th>
<th>Cases (no=75)</th>
<th>Control (no=25)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>13.3</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>65</td>
<td>86.7</td>
<td>24</td>
</tr>
</tbody>
</table>

\[ X^2 = 0.9 \quad P = >0.05 (0.4) \]

Table (3): Results of ELISA-IgM according to the status of pregnancy

<table>
<thead>
<tr>
<th>ELISA-IgM</th>
<th>case (n=75)</th>
<th>( X^2 )</th>
<th>P –value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abortion (n=37)</td>
<td>IUFD (n=7)</td>
<td>PTL (n=31)</td>
</tr>
<tr>
<td>Seronegative</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Mild elevation</td>
<td>2</td>
<td>5.4</td>
<td>0</td>
</tr>
<tr>
<td>(17-100 unit)</td>
<td>4</td>
<td>10.8</td>
<td>0</td>
</tr>
<tr>
<td>Moderate elevation</td>
<td>1</td>
<td>2.7</td>
<td>0</td>
</tr>
<tr>
<td>(100 - 300 unit)</td>
<td>7</td>
<td>18.9</td>
<td>0</td>
</tr>
<tr>
<td>Total positive</td>
<td>7</td>
<td>18.9</td>
<td>0</td>
</tr>
</tbody>
</table>
**Table (4):** The relation between serum IgG and serum IgM ELISA in the studied groups

<table>
<thead>
<tr>
<th>Anti- <em>Toxoplasma gondii</em> antibodies</th>
<th>Case No=75</th>
<th>Control No=25</th>
<th>Total No=100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No(%)</td>
<td>No(%)</td>
<td>No(%)</td>
</tr>
<tr>
<td>IgG(+)IgM(-)</td>
<td>30 (40)</td>
<td>6(24)</td>
<td>36(36)</td>
</tr>
<tr>
<td>IgG(-)IgM(+)</td>
<td>6 (8)</td>
<td>0(0)</td>
<td>6(6)</td>
</tr>
<tr>
<td>IgG(+)IgM(+)</td>
<td>4 (5.3)</td>
<td>1(4)</td>
<td>5(5)</td>
</tr>
<tr>
<td>IgG(-)IgM(-)</td>
<td>35 (46.6)</td>
<td>18(72)</td>
<td>53(53)</td>
</tr>
</tbody>
</table>

**Table (5):** The relation between serum IgG& IgM ELISA and histopathological method in the studied groups

<table>
<thead>
<tr>
<th>Histopathological method</th>
<th>serum IgG&amp; IgM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ ve</td>
<td>- ve</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>40</td>
<td>95.2</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>53.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histopathological method</th>
<th>positive serum IgG&amp; IgM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ ve</td>
<td>- ve</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>21.7</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>28</td>
</tr>
</tbody>
</table>
Table (6): The relation between serum IgG & IgM ELISA and immunohistochemical method in the studied groups

<table>
<thead>
<tr>
<th>Immunohistochemical method</th>
<th>serum IgG&amp; IgM</th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ ve</td>
<td>- ve</td>
<td></td>
</tr>
<tr>
<td>Complicated=75</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Positive</td>
<td>40</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>53.3</td>
<td>35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immunohistochemical Method</th>
<th>serum IgG&amp; IgM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ ve</td>
<td>- ve</td>
</tr>
<tr>
<td>Control=25</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>28</td>
</tr>
</tbody>
</table>
Fig. (1): Cut section of the decidua infected with toxoplasmosis showing hemorrhage with lymphocytic infiltration in the stroma (H&E) (x100).

Fig. (2): Cut section of the placenta infected with toxoplasmosis showing dense focal lymphocytic infiltration in the stroma (H&E) (x200).
Fig.(3): Cut section of the decidua infected with toxoplasmosis showing mature neutrophils in the stroma (H&E) (x200)

Figure (4): Positive apoptosis in the villi showing brownish discolor of trophoblastic cells (Fas, x 400).
Discussion:

In the present study, ELISA test was used for detection of *Toxoplasma* IgG, this study shows that total positive IgG in studied group was (41%). The difference between the two groups was statistically insignificant (*p* > 0.05).

These results were in agreement with Tammam *et al.*, (2013) in Egypt; Makiani *et al.*, (2012) in Iran; Almushail *et al.*, (2014) in Saudi Arabia and Hamad *et al.*, (2013) in Kurdistan (Iraqi) who reported that the prevalence of IgG was 46.1%, 41.9%, 38.8%, 37.5% respectively among pregnant women by ELISA test.

On the other hand, Eldeeb *et al.*, (2012) in Egypt; Zemene *et al.*, (2012); Gebremedhin *et al.*, (2013) in Ethiopia and Bittencourt *et al.*, (2012) in Brazil found higher prevalence of IgG 67.5%, 81%, 78.4%, 59.8% among pregnant women respectively. This high result was explained as IgG antibodies can remain positive for many months or even years after infection, inadequate hygiene, and suitable climatic factors for survival of oocysts.

On the other hand, Liu *et al.*, (2009) in China and Ertug *et al.*, (2005) in Turkey found lower prevalence 10.6%, 30.1% respectively.

The present study shows that, total positive IgM in studied group was (11%). (*p* > 0.05) not significant. This was in agreement with Al-Mohammad *et al.*, (2010) in Saudi Arabia, Tabbara and Saleh, (2005) in Bahrain and Bobić *et al.*, (2010) in Serbia who reported that the prevalence of IgM was 8.8%, 11.4%,10% respectively among pregnant women by ELISA test. On the other hand, Aqeely *et al.*, (2014) in Saudi Arabia; ElDeeb *et al.*, (2012) in Egypt and Bittencourt *et al.*, (2012) in Brazil found lower prevalence 6.2%, 2.8%, zero respectively.

In the present study prevalence of IgM in complicated pregnancy was 13.3%. Bobić *et al.* (2010) in Serbia supported this 10%. On the other hand ELFakahany *et al.*, (2002) in Egypt found higher prevalence 27.3% while Nimri *et al.*, (2004) in Jordan found lower prevalence 7.4%. This high result was explained as IgM antibodies may persist for > one year after acute infection and physiological changes and, stressful demands and the
general hormonal imbalances associated with pregnancy may lower the resistance of pregnant female to disease.

The discrepancy between the present and previous results may be attributed to the type of studied women, as anti-Toxoplasma IgM seropositivity was higher in women with complicated gestation than those with normal gestation and the sensitivity and specificities of the various commercially available serologic test (Wilson et al., 1997).

In the current study, the examined sections of placenta showed villitis, intervillous placentitis. Inflammatory cells were found in placental sections was mature neutrophils, lymphocytes, histocytes and few plasma cells.

This was supported by Mederle et al. (2008) and Tammam et al. (2013) who reported that villitis due to toxoplasmosis was characterized by chronic inflammatory cells, lymphocytes, histocytes, and few plasma cells in the placental villous stroma.

This study shows that the immunohistochemical examination of the D&C and placental samples of studied groups revealed that, the highest number of cases having apoptosis 27 out of 37 aborted females. While, in control group no female have apoptosis. The difference between the types of complications was statistically significant (p<0.05).

The results of the current study were in agreement with Ali and Ahmed (2008) and Aschkenaziet al. (2002) who found that trophoblastic apoptosis occurred by the FasL/Fas pathway and suggest that FasL expressed on the trophoblasts and activated maternal lymphocytes can induce trophoblast Fas-mediated apoptosis and the increased Fas expression on trophoblasts may make them more sensitive to Fas-mediated apoptosis.

**Conclusion:** ELISA is the important screening test commonly used as they are rapid and easy. But it isn't confirmatory test for diagnosis of infection. Histopathological changes in toxoplasmosis may be suggestive but they are not confirmatory for the diagnosis unless finding the characteristic morphological forms of the parasite. Immunohistochemical method detected apoptotic changes in tissues infected with Toxoplasma which is marked
in acute infection as the parasite causes severe degree of tissue damage, while in chronic infections; the parasite inhibits apoptosis to remain dominant in the cells from which it takes its nutrition. There are no pathognomonic criteria for toxoplasmosis.

Acknowledgment: We would like to acknowledge all members of parasitology, Obstetrics and Gynecology Departments of Benha Faculty of Medicine, Benha University for their help

References:


