ORIGINAL ARTICLE

Role of IgG avidity test in diagnosis of Toxoplasmosis among immunocompromised patients

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ABSTRACT

Background: Toxoplasmosis is an opportunistic life threatening infection in immunocompromised patients caused by Toxoplasma gondii (T. gondii). Objectives: The present study was carried out to detect role of IgG avidity test in diagnosis of toxoplasmosis and distinguish between recent and old infection among immunocompromised patients and to detect risk of transmission of toxoplasmosis by blood donation. Methodology: This case control study was carried upon two groups, Group (1): 100 immunocompromised patients and Group (2): 100 individuals as immunocompetent control group. All serum samples were tested for anti-Toxoplasma (IgG, IgM) by Enzyme linked immunosorbent assay (ELISA) test. IgG avidity ELISA test was done to all cases with positive IgM, positive IgG or both. Results: Seroprevalence rate of anti-Toxoplasma IgM and IgG in immunocompromised group was 11% and 50% respectively which was higher than control group 4% and 32%. Results of IgG avidity test although ten cases (12.2%) were positive IgM and IgG, but they were old infection (avidity index (AI) was > 50%). Also two cases (2.44%) were positive IgM and IgG and they were recent infection (AI was < 45%). Conclusion: Diagnosis of toxoplasmosis should not only relay on results of IgM and IgG ELISA test and must be combined with IgG avidity test as obligatory confirmatory step test for routine serological tests especially in those whom diagnosis of toxoplasmosis is critical as immunocompromised patients.

INTRODUCTION

Toxoplasma gondii (T.gondii) is estimated to infect up to one-third of the world human population. Many studies have detected a wide range of seroprevalence of toxoplasmosis in human varying from 10% to 80%

Immunocompetent individuals are usually asymptomatic, but it is life threatening infection in immunocompromised patients such as patients with HIV, patients with cancer and receiving chemotherapy or organ transplantation patients, due to their lowered immunity, the parasite can transform to an active and tissue damaging tachyzoite form that lead to myocarditis, blindness, and encephalitis and even death.

Blood transfusion is also another important risk of transmission of toxoplasmosis as infection can be transmitted from apparently asymptomatic blood donor particularly if he was at the acute stage of infection.

The traditional diagnosis of toxoplasmosis usually depends on serological tests, Enzyme Linked Immunosorbent Assay (ELISA) remains the most common method applied in clinical laboratories, but IgM and IgG ELISA test is not always sufficient in the diagnosis of early and late toxoplasmosis. Anti-Toxoplasma IgM is the main marker of recent infection, but unfortunately it may produce false positive results as it may persist in the serum for long time after acute infection, which may be due to nascent toxoplasmosis seroconversion, residual IgM or nonspecific IgM reaction.

Simultaneous detection of IgG and IgM in the first serum sample requires additional testing to estimate the date of infection since IgM antibodies may also persist for up to 18 months or longer post infection, T.gondii avidity assay has been proven to be useful as high avidity rules out primary infection occurring in at least the preceding 4 months.

The IgG avidity test denotes to functional affinity that based on antigen and antibody binding forces. During initial stage after primary infection and IgG antibodies bind weakly to the antigen but over weeks and subsequent months antibody-antigen binding forces increases and the avidity increases progressively.

Studies have predicted that combination of both IgG avidity test with traditional ELIZA IgM test improved the diagnosis of toxoplasmosis in differentiation.
between acute and chronic infection when single serum sample is available which is important in immunocompromised patients 10.

This study aimed to detect role of IgG avidity test in diagnosis of toxoplasmosis and distinguish between recent and old infection among immunocompromised patients, to detect difference in seroprevalence rate of toxoplasmosis between immunocompromised patients and immunocompetent individuals and to detect risk of transmission of toxoplasmosis by blood donation.

METHODOLOGY

Study design and Study population:

This case-control study was carried out on two groups, Group (1) consisted of 100 immunocompromised patients recruited from National Liver Institute and Hospitals of Ministry of Health in the period from July 2017 to July 2018 that were divided according to inclusion criteria into three subgroups, group (1a) consisted of 23 patients with malignancy receiving chemotherapy or radiotherapy, group (1b) consisted of 44 patients on hemodialysis therapy and group (1c) consisted of 33 patients with malignancy receiving chemotherapy or radiotherapy, group (1c) consisted of 33 patients who gave no history of blood donation.

Group (2) consisted of 100 immunocompetent individuals as control group matched to immunocompromised group in age and were divided into two subgroups, group (2a) consisted of 55 healthy blood donors and group (2b) consisted of 45 immunocompetent individuals that gave no history of blood donation.

Ethical consideration

This study was approved by the Committee of Research and Ethics of National Liver Institute, Menoufia University. Written informed consent was given by patients and all procedures were explained to them and all the participant names were replaced by code numbers to maintain privacy of the participants.

Questionnaire: Demographic data such as age, gender, occupation and medical history of both groups were recorded.

Samples collection and storage:

Whole blood was collected into closed test tube and was allowed to be clotted by leaving it undisturbed at room temperature for 15-30 minutes then centrifugation for 10 minutes and supernatent serum was immediately transferred into clean tube and all collected samples were stored at -20.

Detection of Anti-T.gondii IgM and IgG by ELISA test:

All samples were tested by ELISA IgM and IgG test (BioCheck, Inc) according to the kits instructions, diluted sera were added to T.gondii antigen-coated microtiter plate and then anti-human antibodies conjugate were added. After incubation and repeated washing, the chromogenic substrate was added, and the optical densities were read by ELISA reader

Serum samples that exhibited positive IgM, positive IgG or both were tested by IgG Avidity ELISA test (Institut Virion\Serion GmbH, Germany kit) according to the kits instructions, 100 µl diluted patient’s serum sample were dispensed into the T.gondii IgG microtiter plate in a duplicate row (row A and row B) then 150 µl avidity reagent were used to elute low-avidity antibodies from the Toxoplasma antigen. After incubation and repeated washing, optical density of each well was read in a microtiter plate reader at 405 nm. IgG avidity was calculated as the percentage of the row A titer and the row B titer and expressed as Avidity Index (% = (Optical density (OD) of the sample treated with avidity reagent / (OD of the sample treated without avidity reagent) × 100 and obtained results was categorized into three groups: low avidity, lower than 45; high avidity, above 50 and border line, equal or lower than 50 and equal or higher than 45.

Statistical analysis:

Data were collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis, (version 20; Inc., Chicago, IL). Chi- square test, Fisher exact test and Student t-test were used for analytical statistics.

Probability value: P-value ≤ 0.05* means significant and P-value > 0.05 means non-significant.

RESULTS

Out of 100 patients in immunocompromised group (11%) were seropositive for Toxoplasma IgM, which was higher than that in immunocompetent control group (4%) and the difference was statistically non-significant (p >0.05). Regarding results of seroprevalence rate of IgG, out of 100 patients in immunocompromised group (50%) were seropositive which was also higher than seroprevalence rate in immunocompetent group (32%) and the difference was statistically significant (P-value=0.01)(table 1).
Table (1): Statistical analysis of anti-Toxoplasma antibodies detected by ELISA IgM and IgG among studied groups.

<table>
<thead>
<tr>
<th>Type of patient</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Total</th>
<th>Chi-Square</th>
<th>P-value</th>
<th>Odds Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM Negative</td>
<td>89</td>
<td>96</td>
<td>185</td>
<td>3.532</td>
<td>0.06</td>
<td>2.966(0.911-9.655)</td>
</tr>
<tr>
<td>Positive</td>
<td>11</td>
<td>4</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG Negative</td>
<td>50</td>
<td>68</td>
<td>118</td>
<td>6.697</td>
<td>0.01</td>
<td>2.125(1.196-3.775)</td>
</tr>
<tr>
<td>Positive</td>
<td>50</td>
<td>32</td>
<td>82</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Group 1=immunocompromised group & Group 2= immunocompetent control group.

Table (2): Statistical analysis of anti-Toxoplasma antibodies detected by ELISA IgM and IgG among different subtypes of group (1).

<table>
<thead>
<tr>
<th>Group (1)</th>
<th>Subtypes of group (1)</th>
<th>Test of significance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cancer group</td>
<td>Dialysis group</td>
<td>Transplantation group</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>38</td>
<td>29</td>
</tr>
<tr>
<td>IgM Negative</td>
<td>95.7%</td>
<td>86.4%</td>
<td>87.9%</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>6</td>
<td>12.1%</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>IgG Negative</td>
<td>43.5%</td>
<td>68.2%</td>
<td>30.3%</td>
</tr>
<tr>
<td>Positive</td>
<td>56.5%</td>
<td>31.8%</td>
<td>69.7%</td>
</tr>
</tbody>
</table>

* =fisher exact test & ** =chi square test

Table (3): Statistical analysis of anti-Toxoplasma IgM and IgG antibodies detected by ELISA among subtypes of group (2).

<table>
<thead>
<tr>
<th>Group (2)</th>
<th>Subtypes of group (2)</th>
<th>Test of significance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood donors group</td>
<td>Non blood donors group</td>
<td>Total</td>
</tr>
<tr>
<td>IgM Negative</td>
<td>51</td>
<td>45</td>
<td>96</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>IgG Negative</td>
<td>37</td>
<td>31</td>
<td>68</td>
</tr>
<tr>
<td>Positive</td>
<td>18</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>
IgG avidity test was used as a confirmatory test to discriminate between recent and old infection. Although ten cases (12.2%) were positive IgM and positive IgG (by IgM, IgG ELISA test), but their avidity index was > 50% which indicate old infection. But also, two cases (2.44%) were positive IgM and positive IgG and their avidity index was <45% which indicate recent infection. In addition to only one case (0.8%) was positive IgM only and was borderline infection (its avidity index 45-50%) and the difference was statistically non-significant (p >.05%) and 56 case (68.29%) was positive IgG only and were old infection (avidity index >50%), but 13 cases (15.85%) were positive IgG only and were recent infection. Only one case (1.22%) was positive IgG only and borderline infection and the difference was statistically highly significant (p value<.05%) (table 4).

Table (4): IgM ELISA & IgG ELISA test versus avidity IgG ELISA test.

<table>
<thead>
<tr>
<th>IgM</th>
<th>Avidity index</th>
<th>IgG</th>
<th>Total</th>
<th>Fisher exact test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative IgM, IgG</td>
<td>115</td>
<td>0</td>
<td>115</td>
<td>62.2%</td>
</tr>
<tr>
<td></td>
<td>Acute infection</td>
<td>0</td>
<td>13</td>
<td>13</td>
<td>7.0%</td>
</tr>
<tr>
<td></td>
<td>Borderline infection</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.5%</td>
</tr>
<tr>
<td></td>
<td>Old infection</td>
<td>0</td>
<td>56</td>
<td>56</td>
<td>30.3%</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acute infection</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>13.3%</td>
</tr>
<tr>
<td></td>
<td>Borderline infection</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6.7%</td>
</tr>
<tr>
<td></td>
<td>Old infection</td>
<td>2</td>
<td>10</td>
<td>12</td>
<td>80.0%</td>
</tr>
</tbody>
</table>

*Avidity index <45%=acute infection, avidity index>50%=old infection and avidity index 45-50%=borderline infection.

**DISCUSSION**

IgM and IgG ELISA test is not always sufficient for diagnosis of early and late toxoplasmosis, because *Toxoplasma*-specific IgM antibodies may persist for 18 months after acute acquired infection\(^8\), so no laboratory test performed alone is enough for diagnosis of toxoplasmosis and combination of conventional serological tests(IgM and IgG levels) and IgG avidity test is mandatory for diagnosis of toxoplasmosis especially in immunocompromised patients\(^{11}\).

In our study, seroprevalence rate of anti-*Toxoplasma* IgM was higher in immunocompromised group (11%) than immunocompetent group indicating that immunocompromised patients were more risky for catching infection and developing acute toxoplasmosis. Seroprevalence rate of IgG was also higher in immunocompromised group (50%) than immunocompetent group (32%) with statistically significant difference (P-value=0.01) indicating that immunocompromised patients are more risky for flaring up of latent infection lead to life threatening toxoplasmosis.

This was similar to the results of global meta-analysis study that also detected higher seroprevalence of *T.gondii* in immunocompromised patients (35.9%) than healthy control individuals (24.7%) with P-value< 0.001\(^{12}\).

In both immunocompromised and immunocompetent control groups seroprevalence of anti-*Toxoplasma* IgM and IgG were higher in males than females, regarding age groups the highest percentage of positive cases were in age groups 40-60 years old (the difference was statistically not significant), this may be due to male’s outdoor activities are more than females increasing risk of exposure in males than females and with aging risk of exposure to infection is increasing.

This was in agreement with another study that detected also a higher prevalence rate of toxoplasmosis in males (18%) than females (16%) with no significant differences in gender\(^{13}\), but this was different from another study that detected that seroprevalence rate of toxoplasmosis in females (40%) higher than males (33%) and the age group more than 40 years had the highest prevalence (30%)\(^{14}\).

In contrast to another study, seroprevalence rate of toxoplasmosis in males (50.1%) was almost equal to females (49.9%) but was in agreement with the seroprevalence of toxoplasmosis that was the highest in age group 20 to less than 40 year (p > 0.05)\(^{15}\).

In the present study, seropositive cases for anti-*Toxoplasma* IgM and IgG among different
immunocompromised subgroups were respectively (12.1%) & (69.7%) in liver transplantation, (13.6%) & (31.8%) in dialysis group and (4.5%) & (56.5) in cancer group, the difference was statistically significant

Similarly, seroprevalence rate of anti-Toxoplasma IgG and IgM antibody among renal transplant patients with ELISA technique which was 54% and 4% respectively16. Regarding hemodialysis group seroprevalence, another study also detected higher percentage of anti-Toxoplasma antibodies by latex agglutination test in hemodialysis patients (73.3%) than healthy control group (18%) with a statistical significance (p<0.05) and recommended to do serological screening for all patients on hemodialysis therapy to prevent dissemination of infection through the hemodialysis procedure17. In contrast to another study that detected seroprevalence of T.gondii Infection in hemodialysis Patients with Chronic Renal failure and risk factors in Iraq where seroprevalence of anti-Toxoplasma IgG in hemodialysis patient group was higher (54.1%) than healthy control group (38.2%) and the difference was significant between the two groups [P-value= 0.0465], but anti-Toxoplasma IgM were not detected neither in the patients nor the healthy subjects18.

Another study also detected higher seroprevalence of anti-Toxoplasma IgM (4%) among cancer patients than control group (2%) but the difference was statistically not significant and detected also higher seroprevalence of anti-Toxoplasma IgG (20%) than control group (8%) and the difference was statistically significant19. Similarly, higher seroprevalence rate of toxoplasmosis was detected in patients with cancer (20.59%) than that without cancer (6.31%) and the difference was statistically significant (p-value=0.001)19. In contrast to another study, lower seroprevalence rate of anti-Toxoplasma IgG (41.51%) was detected and T.gondii IgM antibody was not detected at all in patients with malignancy20.

In this study seroprevalence rate of anti-Toxoplasma IgM and IgG antibodies among blood donors was also done that was 7.3% and 31.1% respectively and the difference was statistically not significant and it was consensus with that reported by another study which declared that a high prevalence of infection by T. gondii was found among Brazilian blood donors and the risk of transfusional transmission of T. gondii by blood products increases with the time of parasitemia21.

In another study, out of 380 blood donors, 2(0.5%) were seropositive for anti-T.gondii IgM and 131 (34.47%) were seropositive for anti-T. gondii IgG 22 which was different seroprevalence rate of toxoplasmosis among blood donors than our results.

In the present study although ten cases (12.2%) were positive IgM and positive IgG (by IgM, IgG ELISA test), but their avidity index (AI) was > 50% which indicate old infection. But also, two cases (2.44%) were positive IgM and positive IgG and their AI was <45% which indicate recent infection. In addition to only one case (0.8%) was positive IgM only and was borderline infection (it’s AI 45-50%) and the difference was statistically non-significant (p >.05%) and 56 case (68.29%) was positive IgG only and were old infection (AI>50%), but 13 case (15.85%) were positive IgG only and were recent infection. Only one case (1.22%) was positive IgG only and borderline infection and the difference was statistically highly significant (p value<.05%).

A similar retrospective study also was done for proper serological diagnosis of toxoplasmosis in which IgG avidity test was performed for all IgG positive patients regardless of their IgM seropositivity where low avidity was found in 0.7% of IgM-negative patients’ sera and equivocal avidity was detected in 6.5% and Low avidity was detected in 2.6% of IgM positive patients23.

It was also the same in another study demonstrated that ELISA and IgG avidity together would be the reliable tests for evaluation and confirming diagnosis of toxoplasmosis especially when a single serum sample is only available24. These findings were supported also by another study which stated that although real time PCR based method is a rapid, sensitive, specific and quantitative way of diagnosis of toxoplasmosis especially in very early infection, it is costed and needs well trained personnel and routine application of it is very difficult so serological test especially avidity test is cost effective and can be used as screening test especially in developing country25. In contrast to another study that demonstrated that although IgG avidity test had revolutionized serological diagnosis of toxoplasmosis, no laboratory test performed alone is self-sustained and combinations of serological, culture-based and PCR techniques must be performed26.

**CONCLUSION**

Diagnosis of toxoplasmosis should not only rely on results of IgM and IgG ELISA test alone and must be combined with IgG avidity test as obligatory confirmatory step test for routine serological tests especially in those whom diagnosis of toxoplasmosis is critical as immunocompromised patients.

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- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
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