Assessment of C3 and C4 component of complement system in aborted women infected with Toxoplasma gondii

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Abstract
Background: The complement system consists of more than 30 proteins that are either present as soluble proteins in the blood or present as membrane-associated proteins. Inappropriate complement activation and complement deficiencies are the underlying cause of the pathophysiology of many diseases.

Methods: A case-control study was conducted to assess role of complement system during toxoplasmosis in Najaf province. Thirty five sera of infected women with toxoplasmosis by investigation antibody against toxoplasmosis for IgG and IgM by ELISA technique were tested to determine the levels of C3 and C4 by using single radial immunodiffusion technique and compared with the ten of control sera of non-infected subjects and non-abortion.

Results: The high C3 in significant differences(P<0.05) and C4 with no significant differences(P>0.05) levels were found in aborted toxo-positive women in compared with control. Likewise, it was found that association between concentration of C3,C4 and number of abortion.

Conclusion: complement were play role in immune response of pregnant women especially against toxoplasmosis that cause abortion to these women.

1. Background
Toxoplasmosis is one of the most important worldwide diseases, caused by Toxoplasma gondii. Primary infection in an immunocompetent person is usually asymptomatic [1]. Toxoplasmosis is important due to the possibility of transplacental transmission and harming the fetus [2]. If the mother is infected in the first trimester, may lead to abortion, stillbirth or severe disease of fetus [3].

The complement system helps or complements the ability of antibodies and phagocytic cells to clear pathogens from an organism. It is part of the immune system called the innate immune system [4].

Initially, complement was thought to play a major role in innate immunity where a powerful and rapid response is mounted against invading pathogens. However, recently it is becoming increasingly evident that complement also plays an important role in adaptive immunity involving T and B cells that help in elimination of pathogens [5, 6].
It was observed that B cells could bind C3, subsequently, it was recognized that the complement receptors CR1 and CR2 mediate complement-associated B cell functions. These receptors are expressed on B cells, and on a subset of T cells [7, 8].

The role of complement at the placenta tissue like in any other tissue in the body is to protect both the fetus and the mother against infectious and other toxic agents [9]. Uncontrolled complement activation is prevented in successful pregnancy by three regulator protein DAF, MCP and CD59 positioned on the surface of the trophoplasts. Extensive complement activation in the placenta places the fetus at risk for growth restriction or death [10].

Activation of complement by parasites is involved in several mechanisms of the host parasite relationship. In most of the experiments performed in vitro, complement activation was found to be lethal for the parasites [11]. The studies that studied association between Toxoplasma and complement system are very few, accordingly, we carried out this study which aimed to investigate the impact of Toxoplasma infection on serum level of C3 and C4 and association of these parameters with pathogenesis of toxoplasmosis including abortion.

2. Methods

2.1. Study design

This case-control study was performed on 45 women, The mean age of the participant women were 29.5±7.6 (range:16-50) years. The women in the current study were classified into two groups; First group: involved 35 women with bad obstetrician history (BOH), who suffered from previous abortions, these samples considered as a positive sera by using Toxo-IgM ELISA and Toxo-IgG ELISA. Second group: included 10 non aborted women without obstetric problems and had more than one successful birth who negative by Toxo-IgM and Toxo-IgG ELISA. They were attending AL-Zahraa Maternity and Pediatric Teaching Hospital, Najaf City/Iraq.

This study was approved before its commencement by the ethical committee of the Faculty of Medicine, University of Kufa, and informed consent was obtained from all individuals.

2.2. Sample collection

Four milliliter of venous blood was collected from patients and control in sterile serum tube and left for one hour at room temperature to allow the clot to form. Then, centrifuged at 3000 rpm for 15 min. to separate the serum which divided in eppendorf tubes (200μl) and kept at deep freeze (-20 C˚) until used.

2.3. Immunological tests

The search for anti-T. gondii antibodies in serum was performed by Enzyme Linked Immunosorbent Assay (ELISA) by using Toxo IgM ELISA and Toxo-IgG ELISA (Human-Germany). It was done according to manufacturer’s instructions

2.4. Radial immune diffusion plate for determination C3 and C4 protein

The test is performed by using (RID) kit (LTA –Italy). The plate was removed from its envelope and leaved to stand at room temperature for few minutes so that any condensed water in the wells was evaporated. Then the wells were filled with 5μl of samples and controls and waited they have been completely adsorbing before handling the plate. The plate was closed and waited the required incubation period 72 hour. Measured the precipitating ring around the well after incubation and compared with conversion table that provided with the kit. However the normal value of C3 according to WHO is 91-156 mg/dl. And of C4 is 20-50mg/dl.

2.5. Statistical analysis

Data of the studied group were checked for any error or inconsistency, entered and analyzed by using the statistical package for social sciences
Seroprevalence data obtained are shown in figure (1). It had been significantly found the positive toxoplasmosis cases (35) among aborted women (IgM positive and IgG positive) in comparison to (0) in non aborted women (P<0.05).

3. Results

3.1. Immunological tests

![Figure 1: Distribution of Toxo-IgM, Toxo-IgG among aborted and control.]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aborted toxo positive (n=35)</td>
<td>Control (n=10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>C3</td>
<td>Low</td>
<td>8</td>
<td>22.9</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>11</td>
<td>31.4</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
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<td>45.7</td>
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<tr>
<td></td>
<td>Total</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>C4</td>
<td>Low</td>
<td>13</td>
<td>37.2</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>4</td>
<td>11.4</td>
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<td>18</td>
<td>51.4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>35</td>
<td>100</td>
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</table>
Table 2: Association of C3 and C4 complement components levels according to number of abortion

<table>
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<tr>
<th>Parameter</th>
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<th>2</th>
<th>More than 2</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>C3</td>
<td>Low</td>
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<td>0</td>
<td>2</td>
<td>11.8</td>
<td>6</td>
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<tr>
<td></td>
<td>High</td>
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<td>12.5</td>
<td>9</td>
<td>52.9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>7</td>
<td>87.5</td>
<td>6</td>
<td>35.3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>8</td>
<td>100</td>
<td>17</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>C4</td>
<td>Low</td>
<td>2</td>
<td>25</td>
<td>4</td>
<td>23.6</td>
<td>7</td>
</tr>
<tr>
<td></td>
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<td>0</td>
<td>3</td>
<td>17.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
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<td>75</td>
<td>10</td>
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</tr>
<tr>
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<td>100</td>
<td>17</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

4. Discussion

The results of present study showed that there are significant differences in concentration of C3 among women with Toxo-IgM positive in comparison with control group. This finding in accord with the results by other researches, [12] and [13] mentioned that the highest level of C3 in women with positive anti Toxoplasma IgM while the lowest level of this component was in women with negative anti Toxoplasma IgM. [14] reported that, significant differences were reported in the levels of C3 between the serum of infected women in comparison with no infected one.

Complement protein C3 is a central molecule in the complement system, whose activation is essential for all the important functions performed by this system. C3 is the highest concentration in the serum which is activated commonly by classical and alternative pathway; the activation of C3b by C4b2b is a major step in the complement activation process because each C4b2b complex can activate as many as 200 C3 molecules [15].

C3 promotes phagocytosis, supports local inflammatory responses against pathogens, and directs the adaptive immune response to select the appropriate antigens for a humoral response; however, its unregulated activation leads to host cell damage [16].

The present findings differ from the results of [17] who found no significant differences of C3 level between patients and controls. The same researcher found no significant differences were observed in the serum mean levels of complement components C4 between patients and controls, and this result come agree with current result.

However the present study in consistent with [12,13] who mentioned that the highest level of C4 in women with positive anti Toxoplasma IgM while the lowest level of C4 was in women with negative anti Toxoplasma IgM, and [14] reported that, significant differences were reported in the levels of C4 between the serum of infected women in comparison with no infected one.

Further analysis between concentration of C3,C4 and number of abortion in aborted women with Toxo-IgM positive revealed that there are association between concentration of C3,C4 and number of abortion.

[13] showed that the complements level (C3, C4) varies among pregnant women with...
different history of abortion (one or two abortion) and presence of anti *Toxoplasma*. These findings confirm the role of complement system in immune response against *Toxoplasma gondii* infection that cause abortion in pregnant. In this context, Antibody and complement will lyse tachyzoites in vitro via the classical pathway [18].

5. Conclusion:
Complement system may play a good role in immune response against toxoplasmosis in aborted women.

References