Prevalence of Toxoplasma gondii and Cytomegalovirus in Sera of Aborted Women in Zawia City

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ABSTRACT
Toxoplasmosis is caused by the parasite Toxoplasma gondii. This disease is widespread and is considered a zoonotic disease. Infection with this disease leads to stillbirth, miscarriage, or a birth defect during pregnancy. Aim of the study is to determine the seroprevalence of Toxoplasma gondii in the city of Zawia. Over ten months, two hundred and eleven serum samples were collected from pregnant women to be examined for Toxoplasma gondii and cytomegalovirus at Al-Zawiya Hospital, Al-Zawiya city, Libya, and the Toxoplasma gondii parasite and cytomegalovirus were examined. The majority of aborted women were in the age group of 21-30 years (53.1%), followed by the 31-40 years age group (31.7%). Toxoplasmosis infection was more prevalent (32.7%) than cytomegalovirus (CMV) infection (15.2%), with a relatively low co-infection rate (6.6%). IgG (past/chronic infection) was present in 50.7% of cases, and IgM (recent/acute infection) in 59.2%. CMV: IgG was present in 35.5% of cases, and IgM in 69.7%. No significant association between toxoplasmosis IgG and CMV IgG. Significant association between toxoplasmosis IgG and CMV IgM, indicating a relationship between past/chronic toxoplasmosis and recent CMV infection. Highly significant association between toxoplasmosis IgM and CMV IgG, suggesting a link between recent toxoplasmosis and past/chronic CMV infection. Highly significant association between toxoplasmosis IgM and CMV IgM, indicating a strong relationship between recent infections of both pathogens. A serological screening program for early detection of toxoplasmosis and cytomegalovirus (CMV) infection in pregnant women and aborted women in Libya is considered essential to avoid all causes that pose problems for women.

INTRODUCTION
Toxoplasma gondii (T. gondii) is one of the most important parasites of medical importance [1,2]. It is found in almost all warm-blooded vertebrates, including humans, and classifies humans globally as an intermediate host. At the same time the final host are cats, rodent (Ctenodactylus gundi) and others [3]. Approximately 30% of the world's population is infected with Toxoplasma gondii [4], and it is acquired mainly through food and water contaminated with parasite eggs released from infected cats when eaten raw or undercooked meat containing cysts of these residues [3]. Toxoplasma gondii infections are worldwide, [5]. It ranks among the highest ranked foodborne parasites globally, third among all foodborne pathogens in the United States, and second among foodborne parasites in Europe [6,7]. In 1908, the world, Nicole and Manceaux, became acquainted with Toxoplasma gondii. This parasite was found in many...
countries such as Africa, Europe, Asia, North and South America, and others. [8]. In 1908, the world, Nicole and Maneaux, became acquainted with Toxoplasma gondii. This parasite was found in many countries such as Africa, Europe, Asia, North and South America, and others. Studies have shown that about 30% - 40% of Iranian people are infected with toxoplasma [9,10].

Toxoplasmosis is ranked fourth among more than 24 pathogens transmitted by ingested food, according to the classification of the Food and Agriculture Organization and the World Health Organization [11,12]. Many diseases, such as blindness, mental retardation, epilepsy, and death, are transmitted as a result of infection with T. gondii to the fetus [13]. Problems increase with infected patients, those with weak immunity, and those suffering from chorioretinitis.[14].

Human cytomegalovirus (CMV) is among the elements that cause severe congenital infections (90% of cases) that lead to severe damage to the fetus during pregnancy and sometimes miscarriage and death. Cytomegalovirus infection throughout pregnancy is extremely complicated [15]. As a high concentration of IgG leads to an infection that is not recent, although the results of a low concentration do not indicate that the infection is recent or old [16]. G (IgG) Through many studies, this type is considered the tool to differentiate between recurrent and acute infection with the parasite. The reason for T. gondii infection is that the antibody has matured (T. gondii IgG), as a result of the slow response to the infection, especially in patients who visit clinics. Compared to patients who do not visit clinics and therefore do not undergo treatment [17,18]. The concentration of antibodies may be low within a year and therefore it cannot be used to detect Toxoplasma gondii infection [19]. Detection of HCMV antibodies is the most commonly used method to identify individuals infected with HCMV. ELISA is the most commonly used test using serum and initial infection M (IgM) for an identifiable virus. for several months, when there is a positive result for IgG antibodies, this is a sign that there was a previous infection with T. gondii and CMV, and this does not mean the current infection [20]. This study aims to provide a study on abortion in the city of Zawia, Libya, with the detection of toxoplasma disease, and cytomegalovirus (CMV) among aborted women Attending Zawia University Hospital and some specialized clinics.

METHODS
Study setting
During ten months, from December 2022 to September 2023, a total of 211 women attending Al-Zawiya General Hospital and Ibn Al-Nafis Specialized Clinic in Zawia, Libya were included in this study. The ages of the study group ranged from ≥12 to ≥46. Blood samples were collected sterilly sterile 5 ml disposable tubes. The samples were named and they are delivered in cold 10C containers to the laboratory. serums they were separated by centrifugation for 5 minutes at 3000 rpm, ans store at 20°C until tested.

Sample collection and serological tests
5 ml of blood was taken and placed in a clotting tube. Sera were separated and stored in sterile tubes at −20°C until serological tests were performed. Sera were screened for IgM and IgG antibodies against Toxoplasma gondii and cytomegalovirus by (VIDAS Toxo IgM and IgG, Biome Rieux, France) according to the manufacturer's recommendations.

Statistical analysis
All statistical analysis was undertaken using software for biostatistics analyses (SPSS).

RESULTS
Table 1 show the distribution of aborted women according to age groups in the Zawiya region of western Libya. The majority of aborted women fall within the 21-30 age group, accounting for 53.1% of the total. This is followed by the 31-40 age group at 31.7%, and the 41-50 age group at 10.9%. Also, it can be noted that women aged 20 and below had the lowest percentage of abortions at 4.3%.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. of aborted women</th>
<th>% of aborted women</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20</td>
<td>9</td>
<td>4.3</td>
</tr>
<tr>
<td>21-30</td>
<td>112</td>
<td>53.1</td>
</tr>
<tr>
<td>31-40</td>
<td>67</td>
<td>31.7</td>
</tr>
<tr>
<td>41-50</td>
<td>23</td>
<td>10.9</td>
</tr>
</tbody>
</table>
Table 2 presents the seropositivity of different infection agents among women in the Zawia region of western Libya. The data shows that 32.7% of the women tested positive for toxoplasmosis, while 15.2% tested positive for CMV (Cytomegalovirus). Additionally, 6.6% of the women tested positive for both toxoplasmosis and CMV. It’s noteworthy that 45.5% of the women tested negative for both infections. These results suggest that toxoplasmosis infection was more prevalent than CMV infection among the aborted women in the study population. The co-infection rate was relatively low. However, it's important to note that nearly half of the women did not test positive for either infection, indicating the potential involvement of other factors contributing to the abortions.

**Table 2. Seropositivity of each infection agent**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. of +ve cases</th>
<th>% of +ve cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxoplasmosis</td>
<td>69</td>
<td>32.7</td>
</tr>
<tr>
<td>CMV</td>
<td>32</td>
<td>15.2</td>
</tr>
<tr>
<td>Toxoplasmosis +CMV</td>
<td>14</td>
<td>6.6</td>
</tr>
<tr>
<td>No infections</td>
<td>96</td>
<td>45.5</td>
</tr>
<tr>
<td>Total</td>
<td>211</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3 provides a breakdown of the seroprevalence of IgG and IgM for toxoplasmosis and CMV infections among aborted women in different age groups. The data shows that among women aged 20 and below, 2 (0.9%) tested positive for anti-toxoplasmosis IgG and 4 (1.9%) tested positive for anti-CMV IgG. In the 21-30 age group, 60 (28.4%) tested positive for anti-toxoplasmosis IgG and 36 (17.1%) tested positive for anti-CMV IgG. For IgM antibodies, the percentages were higher across all age groups. These findings reveal varying seroprevalence rates of toxoplasmosis and CMV infections among aborted women in different age brackets. The higher prevalence of IgM antibodies compared to IgG antibodies suggests that a significant portion of these women had been exposed to these infections in the past.

**Table 3. Seroprevalence IgG and IgM of Toxoplasmosis and CMV infections among aborted women.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Total No.</th>
<th>No. of +ve anti- toxoplasmosis Ig'</th>
<th>No. of +ve anti- CMV Ig'</th>
<th>No. of –ve Anti Toxo &amp; Anti CMV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG %</td>
<td>IgM %</td>
<td>IgG %</td>
</tr>
<tr>
<td>20≤</td>
<td>9</td>
<td>2</td>
<td>0.9</td>
<td>4</td>
</tr>
<tr>
<td>21-30</td>
<td>112</td>
<td>60</td>
<td>28.4</td>
<td>65</td>
</tr>
<tr>
<td>31-40</td>
<td>67</td>
<td>37</td>
<td>17.5</td>
<td>42</td>
</tr>
<tr>
<td>41-50</td>
<td>23</td>
<td>8</td>
<td>3.8</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>211</td>
<td>107</td>
<td>50.7</td>
<td>125</td>
</tr>
</tbody>
</table>

Table 4 presents data on the relationship between the presence of IgG antibodies for toxoplasmosis and cytomegalovirus (CMV) infections among the aborted women. 36 cases (17.1%) were positive for both toxoplasmosis and CMV IgG antibodies. 71 cases (33.6%) were positive for toxoplasmosis IgG but negative for CMV IgG. 39 cases (18.5%) were negative for toxoplasmosis IgG but positive for CMV IgG. 65 cases (30.8%) were negative for both toxoplasmosis and CMV IgG antibodies. The p-value of 0.559 is greater than the commonly used significance level of 0.05, indicating that the observed differences in the distribution of cases across the four categories (positive/negative for each infection) are not statistically significant. In other words, based on the chi-square test, there is no significant association between the presence of toxoplasmosis IgG and CMV IgG antibodies in the aborted women included in the study.

**Table 4. Association between Toxoplasmosis (IgG) and Cytomegalovirus (IgG)**

<table>
<thead>
<tr>
<th>Toxoplasmosis (IgG)</th>
<th>Cytomegalovirus (IgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Count</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>36</td>
</tr>
<tr>
<td>Negative</td>
<td>39</td>
</tr>
<tr>
<td>Chi Square</td>
<td>0.342</td>
</tr>
<tr>
<td>P-value</td>
<td>0.559</td>
</tr>
</tbody>
</table>
Table 5 presents data on the relationship between the presence of IgG antibodies for toxoplasmosis and IgM antibodies for cytomegalovirus (CMV) among the aborted women. 85 cases (40.3%) were positive for toxoplasmosis IgG and CMV IgM antibodies. 22 cases (10.4%) were positive for toxoplasmosis IgG but negative for CMV IgM. 62 cases (29.4%) were negative for toxoplasmosis IgG but positive for CMV IgM. 42 cases (19.9%) were negative for both toxoplasmosis IgG and CMV IgM antibodies. The p-value of 0.002 is less than the commonly used significance level of 0.05, indicating that the observed differences in the distribution of cases across the four categories (positive/negative for each antibody type) are statistically significant. In other words, based on the chi-square test, there is a highly significant association between the presence of toxoplasmosis IgG and CMV IgM antibodies in the aborted women included in the study. The presence of IgM antibodies for CMV suggests a recent or acute infection, while the presence of IgG antibodies for toxoplasmosis indicates a past or chronic infection. The significant association between these antibody types implies that there may be a relationship between recent CMV infection and past or chronic toxoplasmosis infection in the context of abortions in this study population.

![Table 5. Association between Toxoplasmosis (IgG) and Cytomegalovirus (IgM)](https://journal.utripoli.edu.ly/index.php/Alqalam/index.png)

Table 6 presents the relationship between the presence of IgM antibodies for toxoplasmosis and IgG antibodies for cytomegalovirus (CMV) among the aborted women. 28 cases (13.3%) were positive for toxoplasmosis IgM and CMV IgG antibodies. 97 cases (46.0%) were positive for toxoplasmosis IgM but negative for CMV IgG. 47 cases (22.3%) were negative for toxoplasmosis IgM but positive for CMV IgG. 39 cases (18.5%) were negative for both toxoplasmosis IgM and CMV IgG antibodies. The p-value of less than 0.001 is much lower than the commonly used significance level of 0.05, indicating that the observed differences in the distribution of cases across the four categories (positive/negative for each antibody type) are highly statistically significant. In other words, based on the chi-square test, there is a highly significant association between the presence of toxoplasmosis IgM and CMV IgG antibodies in the aborted women included in the study. The presence of IgM antibodies for toxoplasmosis suggests a recent or acute infection, while the presence of IgG antibodies for CMV indicates a past or chronic infection. The highly significant association between these antibody types implies that there may be a strong relationship between recent toxoplasmosis infection and past or chronic CMV infection in the context of abortions in this study population.

![Table 6. Association between Toxoplasmosis (IgM) and Cytomegalovirus (IgG)](https://journal.utripoli.edu.ly/index.php/Alqalam/index.png)

Data in table 7 presents on the relationship between the presence of IgM antibodies for toxoplasmosis and IgM antibodies for cytomegalovirus (CMV) among the aborted women. 112 cases (53.1%) were positive for both toxoplasmosis IgM and CMV IgM antibodies. 13 cases (6.2%) were positive for toxoplasmosis IgM but negative for CMV IgM. 35 cases (16.6%) were negative for toxoplasmosis IgM but positive for CMV IgM. 51 cases (24.2%) were negative for both toxoplasmosis IgM and CMV IgM antibodies. The p-value of less than 0.001 is much lower than the commonly used significance level of 0.05, indicating that the observed differences in the distribution of cases across the four categories (positive/negative for each IgM antibody type) are highly statistically significant. In other words, based on the chi-square test, there is a highly significant association between the presence of toxoplasmosis IgM and CMV IgM antibodies in the aborted women included in the study. The presence of IgM antibodies for toxoplasmosis suggests recent or acute infections for these two pathogens. The highly significant association between these IgM antibody types implies that there may be a strong relationship between recent toxoplasmosis infection and recent CMV infection in the context of abortions in this study population. The high percentage of cases (53.1%) that were positive for both toxoplasmosis IgM and CMV IgM antibodies suggests that co-infection with these two pathogens during recent or acute stages of infection may be a significant factor contributing to abortions in this study population.
DISCUSSION
The present study targeted 211 blood samples from different locations, and in the Zawiya region, examined for the presence of antibodies of T. gondii and CMV. We found that the highest percentage of women having abortions is in the age group between 21-30 years (53.1%). These results are consistent with many studies in Iraq that showed a high rate of miscarriage in the age group between 20-30 years [6,14]. On the other hand, a study in America, indicated that the risk of Toxoplasma gondii infection increased with age [13].
Through this study, the percentage was 32.7% of the infections were toxoplasmosis, while 15.2% were infections with cytomegalovirus, while infection with both toxoplasmosis and cytomegalovirus was 6.6%. Many studies indicate the similarity between this study and other studies, that there is a relationship between Toxoplasma gondii, cytomegalovirus, and miscarriage in women. This is consistent with many studies that indicate that prenatal infection is common in terms of its association with congenital malformations and deaths, especially in low-income countries (LMIC) as well as middle-income countries. As for high-income countries, they have improved services for maternal and child health in order to prevent infection after the intervention of (WHO), which emphasized the necessity of prenatal and postnatal examination, and the examination must be sufficient [21, 22]. The number of mothers infected with Toxoplasma gondii reported in this study is high at this time, and work must be done to improve maternal health before and after birth, especially in low- and middle-income countries. Sometimes the infection in the first months leads to the death of the fetus or even reaching the stage of miscarriage, but in the case of the last months it may lead to the birth of children without any kind of symptoms at first at birth, but this does not negate the appearance of symptoms after that because the symptoms may appear later in the coming years [23]. Of the approximately 90% of pregnant women who become infected, they may not show symptoms [24].
Infection with human cytomegalovirus (CMV) is considered one of the elements of infection, and sometimes infection with this virus reaches 90% of cases, which leads to severe damage to the fetus, and sometimes miscarriage or death occurs. It should be noted that infection with CMV throughout pregnancy is complex, as the rate of infection varies. The fetus: 33% to 75% as women advance in pregnancy, and up to 50% if the infection continues throughout the first period of pregnancy[15]. In this study, IgM and IgG antibodies against Toxoplasma gondii and cytomegalovirus were detected. The study showed 28.4% of participants were positive for Toxoplasma IgG, 30.8% for IgM, 17.1% for CMV IgG, and 35.1% for IgM. This study agreed with the study conducted in the city of Al-Bayda, Libya. The sample tested had the highest percentage of CMV IgG (96.36%) and Toxoplasma IgG (39.39%). This study indicated that Toxoplasma and cytomegalovirus infection during pregnancy causes fetal loss [25].
The percentage of Toxoplasma gondii IgM in our study was Was Higher than other results 3.0% [25], 8.3% [26] in Iraq 54% [27] 45.2%. The high rates of T. gondii and CMV infection among miscarriage women may be due to their differences with other studies according to different countries or different places within the same country, social characteristics, economic status, eating habits, contact with animals or cats, climatic conditions, hygiene, or educational level [28].
In this study, it was observed that the highest distribution of IgG and IgM specific for Toxoplasma gondii and cytomegalovirus (CMV) is due to the age groups of mothers, in the age group between 20-30 years. This result is consistent with a study [29]. The reason for this result may be due to the issue of hygiene, low standard of living, eating uncooked meat, and dealing with pets, especially cats. All of these factors may increase the chance of exposure to these microbial agents [28,30].

CONCLUSION
The study revealed a significant prevalence of toxoplasmosis and CMV infections among aborted women in the Zawiya region, with a high proportion of recent or acute infections, particularly in the 21-30 age group. The strong associations between recent and past infections of these two pathogens suggest a potential synergistic effect or co-infection contributing to the abortions. However, it is important to note that a substantial proportion of cases were negative for both infections, indicating the involvement of other factors.
REFERENCES


انتشار طفيل المقوسة الغوندية وفيروس تضخم الخلايا في مصل النساء المجهضات في مدينة الزاوية

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المستخلص

داء المقوسات يسبب طفيل التوكسوبلازما جوندي. وهذا المرض منتشر على نطاق واسع ويعتبر مرضاً حيوانياً. تؤدي الإصابة بهذا المرض إلى وفاة جنين مبكر، أو الإجهاض، أو عيب خلقي أثناء الحمل. لذا، يتم تحديد معدل الانتشار المصري لممرض التوكسوبلازما جوندي في مدينة الزاوية، يعتمد على مدى عشرة أشهر تم جمع ملذتين وأحد عشر عينة مصل من النساء الحوامل لفحصها لممرض التوكسوبلازما جوندي والفيروس الضخم للخلايا في مستشفى الزاوية، مدينة الزاوية. تم فحص نتائج المرض في مدينة الزاوية، ليبيا، وعندما تم فحص طفيل التوكسوبلازما جوندي والفيروس الضخم للخلايا، أظهرت هذه الدراسة أن التوزيع العمري: غالبية النساء المجهضات كن في الفئة العمرية 21-30 سنة (31.7%)، تليها الفئة العمرية 31-40 سنة (31.7%). وانتشار العدوى: كانت عدوى داء المقوسات أكثر انتشارًا (32.7%) من عدوى الفيروس الضخم للخلايا (CMV) (15.2%)، مع معدل منخفض نسبيًا للعدوى المصاحبة (6.6%). ومتلازمة الأجسام المضادة: كان IgG (عديم سابقة/مزمز) موجودًا في 50.7% من الحالات، IgM (عديم سابقة) موجودًا في 35.5% من الحالات، CMV IgG موجودًا في 69.7% والجميع. لا يوجد ارتباط كبير بين داء المقوسات وـ CMV IgM وـ IgG، مما يشير إلى وجود علاقة بين داء المقوسات السباق/المزمن والعدوى الحديثة بـ CMV، مما يشير إلى وجود صلة بين داء المقوسات الحديثة CMV IgG وـ IgM وـ IgG، مما يشير إلى وجود علاقة بين هذه الأمراض، وـ CMV IgM وـ IgG، مما يشير إلى وجود علاقة قوية بين الإصابات الحديثة لكلا المرضين. الاستنتاج: يعتبر برنامج الفحص المصلي للكشف المبكر عن عدوى داء المقوسات والفيروس الضخم للخلايا لدى النساء الحوامل والنساء المجهضات في ليبيا ضرورياً لتجنب جميع الأسباب التي تشكل مشاكل.

الكلمات الدالة: داء المقوسات، الفيروس الضخم للخلايا، الإجهاض، مدينة الزاوية.