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Original Article

Epidemiological aspects of abortion in pregnant women due to *Toxoplasma* and some viral infections

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ABSTRACT

Background: Numerous infections can be passed vertically from a mother to her offspring. Newborn infections are primarily acquired in three ways: through transmission from the mother to the fetus during pregnancy. During delivery through contact with infected genital secretions, blood and feces during the neonatal period through breastfeeding, blood transfusion, and hospital-acquired infections. **Aim:** The aim of this study was to investigate the epidemiological aspects of abortion in pregnant women attributed to *Toxoplasma gondii* assessing the prevalence, and associated risk factors of abortion in pregnant women infected with *Toxoplasma gondii*, *Cytomegalovirus (cmv), Herpes simplex virus (hsv), and Rubella* virus. **Materials and methods:** This cross-sectional study conducted in Kirkuk city from October 10th, 2023, to February 10th, 2024. A total of 120 women with a history of abortion, aged between 20 and 45 years, were enrolled, alongside 60 women with two or more previous normal pregnancies, serving as controls. Inclusion criteria encompassed age between 20 and 45 years and a history of abortion, while exclusion criteria included ectopic or molar pregnancy, chronic diseases, and hormonal treatment during pregnancy. Blood samples were collected, and serological for specific antibodies against *Toxoplasma, Cytomegalovirus* (CMV), H*erpes simplex virus* (HSV), and *Rubella* virus was performed using ELISA kits. **Results:** The study indicated no significant differences in the age distribution and residency between aborted women and the control group. Obstetric properties revealed the majority of aborted women (75.83%) reported 1-2 previous abortions, with variations in the number of children. These findings underscore the importance of comprehensive epidemiological assessment and preventive strategies to mitigate the impact of infectious agents on pregnancy outcomes.

Keywords: *Toxoplasma*, CMV, Abortion, Miscarriage, Seroprevalence

Introduction

Miscarriage is a significant concern in the field of human reproduction. 15% of pregnancies have spontaneous pregnancy loss. It is characterized as the loss of a fetus before 20 weeks of pregnancy or when the fetal weight is below 500 g. Approximately 12-15% of women experience at least one miscarriage during their lifetime [1]. Approximately 5% of women encounter two consecutive pregnancy losses, while 1% experience three or more losses [2]. Numerous infections can be passed vertically from a mother to her offspring. Newborn infections are primarily acquired in three ways: through transmission from the mother to the fetus during pregnancy, during delivery through contact with infected genital secretions, or through the breastfeeding process. TORCH refers to a group of infectious

disorders including Toxoplasma gondii, HIV, syphilis, measles, rubella, CMV, and herpes simplex virus 1 and/or 2. These avoidable contagious diseases pose a public health issue due to their contribution to maternal and neonatal illness, disability, and death. TORCH infections are the primary factors contributing to bad obstetric history (BOH) [3]. The fetus can be infected with the primary infection during pregnancy. Depending on the gestational age of the fetus, it can lead to miscarriage, infection, and silent newborn illness, which is characterized by preclinical but increasing symptoms, particularly affecting the central nervous system and eye [6]. The projected risk of transferring viral and Toxoplasma gondii infections to the baby during the first, second, and third trimesters of pregnancy is predicted to be 15%, 44%, and 71%, respectively. Following the transmission of Toxoplasma gondii through the placenta, the parasite infiltrates specific organs, including immune-privileged areas such as the brain, eyes, and liver. This infiltration can result in miscarriage, stillbirth, or severe complications. Toxoplasma gondii, rubella, CMV, and herpes simplex are significant pathogens that can lead to pregnancy loss in humans [7,8]. The aim of this study was to investigate the epidemiological aspects of abortion in pregnant women attributed to Toxoplasma gondii and assess the prevalence, and associated risk factors of abortion in pregnant women infected with Toxoplasma gondii, cytomegalovirus (CMV), herpes simplex virus (HSV), and rubella virus.

Materials & Methods

A cross-sectional study is carried out in Kirkuk city from 10th of October 2023 to 10th of February 2024 in Kirkuk Teaching Hospital, Gynecological & Pediatirc Hospital and Azadi Teaching hospital in Kirkuk city, Iraq . 120 women with history abortion who age were between 20 and 45 years 60 women who had previous two or more normal pregnancy and with no history of abortion served as control.

The inclusion criteria

1. Age between 20 and 45 years.
2. History of abortion

The exclusion criteria

1. Ectopic or molar pregnancy.
2. Patients with chronic diseases, hypertension, diabetes and thyroid disorders.
3. History of receiving any relevant hormonal treatment during or shortly before the current pregnancy.

Blood collection

Five ml of blood was collected by vein puncture using disposable syringe from each women enrolled in this study. Blood samples were placed into test tubes left for 20-30 minutes at room temperature. Then were centrifuged at 3000 rpm for 5-10 minutes then and the obtained sera were then aspirated using automatic micropipette and transferred into clean Eppendorf tube. Each tube was labelled then stored in deep freeze at -20 °C for serological testing for detecting specific Toxo IgM, Toxo IgG, CMV IgM, CMV IgG, HSV IgM, Rubella IgM, Rubella IgG using ELISA kits as shown in table 1.

Table 1. Kits used in the study

Kit	Source
Toxo IgM ELISA kit	DRG ELISA, Germany
Toxo IgG ELISA kit	DRG ELISA, Germany
CMV IgM ELISA kit	DRG ELISA, Germany
CMV IgG ELISA kit	DRG ELISA, Germany
HSV IgM ELISA kit	DRG ELISA, Germany
HSV IgG ELISA kit	DRG ELISA, Germany
Rubella IgM ELISA kit	DRG ELISA, Germany
Rubella IgG ELISA kit	DRG ELISA, Germany

Toxo: Toxoplasmosis, Ig; Immunoglobulin, CMV: Cytomegalovirus, HSV: Herpes simplex virus.

Enzyme linked to immunosorbent assay

Purified antigen is coated on the surface of micro-wells. Diluted patient serum is added to the wells, and the specific antibody, if present, binds to the antigen. All unbound materials are washed away. Horseradish peroxidase (HRP)-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of Tetramethylbenzidine (TMB) Reagent is added which result in the development of blue color. The blue color turns into yellow color by stopping the enzymatic reaction with sulfurc acid. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of specific antibody in the sample. The results are read by a micro-well reader compared in a parallel manner with calibrator and controls.

Statistical analysis

The SPSS statistical program version 26.1 was used for computerized statistical research. To figure out the chance (P value), Chi square was used for the comparison. When the P value was greater than 0.05, it was statistically significant. When the P value was less than 0.01, it was highly significant, and when it was greater than 0.05, it was not statistically significant.

Results and Discussion

Seroprevalence analysis showed 31.67% positive for *Toxoplasma gondii*, 65% for CMV, 50% for rubella virus IgG, and 30% for HSV IgG among aborted women. Notably, 76.67% of cases showed positive mixed infections of Toxoplasma, CMV, rubella, and HSV. The study compared the general properties of aborted women with those in a control group, assessing age distribution and residency. Surprisingly, there were no significant differences observed in the age composition of aborted women compared to the control group, with 36.67%, 44.17%, and 19.17% of aborted women falling into age groups <30, 30-39, and ≥40 respectively. Similarly, residency distribution showed no notable distinction, with 41.67% of aborted women residing in rural areas and 58.33% in urban areas.

Table 2. General properties of studied women

General properties		Aborted women		Control group		P-value
		No.	%	No.	%	
Age groups	<30	44	36.67	18	30	0.88*
	30-39	53	44.17	31	51.67	
	≥ 40	23	19.17	11	18.33	
	Total	120	100	60	100	
Residency	Rural	50	41.67	29	48.33	0.67
	Urban	70	58.33	31	51.67	
	Total	120	100	60	100	

**P-value >0.05 (non-significant)*

The obstetric properties of the aborted women are outlined in Table 3. Regarding the history of abortion, the majority of aborted women (75.83%) reported having 1-2 previous abortions, while 15% had 3-4, and 9.17% had more than 4 missed abortion. In terms of the number of children, the distribution varies, with 15.83% having one child, 19.17% with two children, 21.67% with three children, and 10% with four children. Notably, 33.33% of the aborted women reported having no children.

Table 3. Obstetric properties of aborted women

		No. of aborted women	Percentage of aborted women
No. of previous abortion	1-2	91	75.83
	3-4	18	15.00
	>4	11	9.17

Total	120	100

Table 4 and 5 presents the seroprevalence of *T. gondii* among 120 aborted women. Among them, 16 (13.33%) tested positive for both *T. gondii* IgM and IgG antibodies, indicating an active infection. Additionally, 22 (18.33%) showed *T. gondii* Toxo IgM negativity and IgG positivity, suggesting a past infection or immunity. The majority, 82 (68.33%), tested negative for both IgM and IgG antibodies, indicating no current or past exposure to *T. gondii*

Table 4. Seroprevalence of *T. gondii* among aborted women (IgM or IgG)

Toxoplasma gondii	No. of aborted women	Percentage of aborted women
IgM and IgG positive	16	13.33
IgM negative /IgG positive	22	18.33
Total positive	38	31.67

Table 5. Seroprevalence of *T. gondii* among aborted women (IgM/IgG)

Toxoplasma gondii	No. of aborted women	Percentage of aborted women
Total IgM and IgG negative	82	68.33
Total	120	100

Table 6 and 7 outline the seroprevalence of Cytomegalovirus among aborted women. Of the total population, 4 (3.33%) tested positive for both CMV IgM and IgG antibodies, indicating an active infection. Additionally, 74 (61.67%) showed CMV IgM negativity and IgG positivity, suggestive of a past infection or the presence of immunity. While 46(38.33%) tested negative for both CMV IgM and IgG antibodies, indicating no current or past exposure to Cytomegalovirus.

Table 6. Seroprevalence of CMV among aborted women (IgM and IgG)

Cytomregalo virus	No. of aborted women	Percentage of aborted women
IgM and IgG positive	4	3.33%
IgM negative /IgG positive	74	61.67%
IgM and IgG negative	46	38.33%
Total	120	100%

Table 7. Seroprevalence of CMV among aborted women (IgM/ IgG)

Cytomregalo virus	No. of aborted women	Percentage of aborted women
IgM and IgG negative	46	38.33%
Total	120	100%

Table 8 presents the seroprevalence of Rubella virus among 120 aborted women, categorized into positive and negative results for Rubella IgM and IgG antibodies. Notably, none of the individuals tested positive for Rubella IgM, indicating the absence of recent Rubella virus infection at the time of testing. However, 60 individuals (50%) tested positive for Rubella IgG, signifying a past infection or vaccination-induced immunity. The other half of the population (60 individuals) tested negative for Rubella IgG.

Table 8. Seroprevalence of Rubella virus among aborted women

Rubella virus	Positive	
	No.	%

IgM	0	0
IgG	60	50

Table 9 illustrates the seroprevalence of Herpes Simplex Virus among 120 aborted women, presenting positive and negative results for HSV IgM and IgG antibodies. No individuals tested positive for HSV IgM, indicating the absence of recent HSV infection at the time of testing. However, 36(30%) tested positive for HSV IgG, suggesting a past infection or the presence of immunity. The remaining 84(70%) tested negative for HSV IgG.

Table 9. Seroprevalence of HSV among aborted women

Herpis simplex virus	**Positive**	
	No.	%
HSV IgM	0	0
HSV IgG	36	30
Total	36	30

Table 10. delineates the seroprevalence of mixed infections among aborted women, focusing on Toxoplasma, CMV (Cytomegalovirus), Rubella, and HSV (Herpes Simplex Virus). Among the 120 cases analyzed, 92 cases (76.67%) showed positive infections of the mentioned pathogens simultaneously, while 28 cases (23.33%) tested negative for such mixed infections.

Table 10. Seroprevalence of mixed infection among aborted women

Mixed infection of Toxoplasma, CMV, Rubella and HSV	**No.**	**%**
Total Positive infection	92	76.67
Total negative	28	23.33
Total number	120	100

Discussion

The study found that 13.33% of 120 women who experienced miscarriage tested positive for both *T. gondii* IgM and IgG antibodies, suggesting an active infection. 18.33% had IgM negativ but IgG positiv, suggesting a previous infection or immunity. Most of them tested negative, which means they had no contact. Al-Azzawy et al. [9] found that IgM antibodies were found in 5.67 percentage of women who had abortions and IgG antibodies were found in 30 percent of cases. Aziz et al. [10] found in study carried out in Kirkuk city which included two hundred (200) aborted women, 28(14%) was IgM positive, While IgG was positive in 66 (33%) .This is similar to what we found. Also, Abdulhasan and Kareem [11] discovered that *T. gondii* was much more common in women who had miscarriages than in the control group, which was made up of women who had no miscarriage. In particular, 59 out of 120 women who had a loss had it happen, which is 49.1% of the total. In the control group, only 5(6.2%) with miscarriage out of 80 women. There were IgG infections in 41.6% of women who had miscarriages, IgM infections in 5%, and IgG and IgM infections together in 2.5%. These results show that *T. gondii* infection and loss are very complicated issues that affect each other. Many of the people in the study had active infections and signs of having been exposed to or being immune to *T. gondii* in the past. This suggests that *T. gondii* may play a part in making pregnancy results worse. Shallal [12] a study in Baghdad City. He found that 48% of women who miscarriaged had IgG Toxoplasma, while only 4% had IgM. The women had a history of spontaneous recurrent abortion. Similarly, Juma and Salman [13] found that 21.67% of women who had a miscarriage at Al-kadhimia Teaching Hospital in Baghdad, Iraq, also had Toxoplasmosis. Compared to other studies done in Iraq and other countries, this study had results that were the same or very similar to those found in Najaf (30.76%), Erbil (29.05%), and Sudan (34.1%). Others, like the one in Diyala, Iraq (38.1%), Iran (41.8%), Turkey (53.3%), and Ethiopia (85.4%), found higher rates for Toxo-IgM (-) / IgG (+), while in the other studies, such as in Malaysia (19.9%) and India (22.40%), found lower rates. So, the Toxo-IgM(+)/IgG(-) rates in our study were lower than those found in Baghdad, Iraq (24.3%), but higher than those found in Diyala, Iraq (2.4%) and Turkey (1%). The rates were higher than those found in Diyala, Iraq (2.4%) but lower than those found in Baghdad, Iraq (24.3%) [14–18]. The minimal variation observed in the rates of *T. gondii* antibodies can be

attributed to the utilization of different techniques and products, each differing in sensitivity and specificity. Conversely, the considerable disparity in rates, both within and between countries, may stem from differences in hygienic practices, socioeconomic status, and cultural factors [19]. The seroprevalence of *T. gondii* in the human population exhibits significant variability among countries, as well as within geographical areas of the same country and among different ethnic groups inhabiting the same region. This widespread distribution of T. gondii is facilitated by the presence of numerous animal hosts capable of harboring and disseminating the parasite [20]. Many research studies have shown that environmental elements like humidity, temperature, diet, and hygiene habits play a big role in these differences in Toxoplasma seropositivity. Many of these external and behavioral factors have a big effect on how the parasite spreads, which in turn changes how common it in different groups of people [20-21]. The study on Cytomegalovirus (CMV) infection in women who had abortions shows a wide range of serostatus, with 3.33 percent showing positive for active infection. A big number of them (61.67%) had negative CMV IgM but positive IgG levels, which that they had been infected or were immune to it in the past. 38.33% tested negative, which means there was no contact or other cause for the pregnancy loss. In line with what we found, Al-Azzawy et al. [9] said that IgM antibodies were found in 5.67% of women who had abortions, IgG antibodies were found in 30% of cases against *T. gondii* , IgG antibodies were found in 26% of cases against CMV, IgG antibodies were found in 24% of cases against HSV, and IgG antibodies were found against measles. Several studies in Iraq and other places have sometimes found similar or very similar results about the Human Cytomegalovirus (HCMV) seroprevalence among women who can have children. One study in Thi-Qar found that 70% of people had seroprevalence. Rates were higher in Iran, the Kingdom of Saudi Arabia, Turkey, India, Benin, Egypt, Gambia, Malaysia, China, Singapore, Spain, and Thailand, where they were 84% to 100%. However, the different ELISA results in these studies could be because they used different ELISA kits from different companies, which have different levels of sensitivity [22–28]. It's interesting that the rate of seropositive HCMV-IgG in this study was higher than in a number of other countries that are usually thought of as developed. The seroprevalence was 30.4% in Ireland, 46.8% in France, 54.4% in the UK, 56.3% in Finland, 56.9% in Australia, 60.0% in the US, and 78.0% in Russia [26–32]. The differences in seroprevalence may be caused by a number of things, including cultural and hygienic habits. In general, the rates of seroprevalence are lower in developed countries. A number of studies from around the world have also shown that HCMV antibodies are more common among women of childbearing age in developing countries and among people with lower incomes than in developed countries [35]. The seroprevalence data in this study about the Rubella virus in 120 women who had abortions shows some interesting trends that help us understand how the infection spreads in this group, the fact that there were no positive results for Rubella IgM antibodies shows that none of the people tested had recently been infected with the Rubella virus. It seems that recent Rubella outbreaks or acute infections were not common among the women who had abortions during the study time [34]. On the other hand, the fact that 50% of people have Rubella IgG antibodies is interesting because it means they have had Rubella before or were immune to it because of a vaccine. This means that a big part of the people in the study had been exposed to the Rubella virus before, either naturally or through a vaccine [35]. The appearance of IgG antibodies shows that the immune system has responded to the Rubella virus, protecting the body from getting infected again. This result shows how important Rubella vaccination programs are for stopping the spread of the Rubella virus and the bad effects that come with it, like congenital Rubella syndrome. No show positive result HSV IgM, which means they had not recently been infected with HSV at the time of testing. But 36 (30%) tested positive for HSV IgG, which means they had an infection or were immune to it. The other 84 (70%) did not test positive for HSV IgG. This was the same finding that was seen in Kirkuk city (35.22%), Waset province (31.3%), Tanzania (33%) and Sweden (34%). Though it was lower than the rates in Turkey (63.1%), Iran (43.75%), and Uganda (86%) [36, 37, 38]. These may have something to do with different cultural and socioeconomic factors. They may also have something to do with HSV-2 being co-infected with other viruses that help it spread and make it more common, especially HIV, which uses the same route of transmission and is common in these areas and may be endemic. The current figures were better than the ones in Saudi Arabia, which were 27.1%. [39] Again, they might have a follow-up scheme that works across the whole country to stop the disease from spreading.

Conclusion

Age distribution and residency did not significantly differ between aborted women and the control group and majority of aborted women had 1-2 previous abortions .Active infections of *T. gondii* were detected in 13.33% of aborted women, and the study showed that 3.33% of aborted women exhibited active CMV infection, while none of the aborted women tested positive for recent *Rubella* virus infection, and recent *HSV* viruse infection.

Recommendations

Implement educational programs to increase awareness among women, especially those of reproductive age, about the risks associated with multiple abortions and the importance of contraceptive methods to prevent unintended pregnancies.

Establish routine screening protocols for *Toxoplasma gondii* and CMV infections during prenatal care visits.

Ethical approval

The native ethics group states that these trials were approved and that all participating patients supplied informed consent and knowledge about the purpose of the investigation. The approval of the Northern Technical College, Kirkuk, and Azadi and Kirkuk Teaching Hospitals was also obtained in document number 581, dated 9/18/2023.

Conflict of Interest

The authors declare that there is no competing of interests.

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