

ORIGINAL ARTICLE

Toxoplasma, Cytomegalovirus and Rubella Infections among Aborted Women Attending Sohag University Hospital, Egypt

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ABSTRACT

Key words:

Toxoplasma, Rubella, Cytomegalovirus, IgM, IgG, Abortion.

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Background: Undesirable fetal outcomes and reproductive failures may follow *Toxoplasma*, *Cytomegalovirus (CMV)* and *Rubella* infections. **Objective:** Detection of *Toxoplasma*, *Rubella* and *CMV* infections among aborted women attending Sohag University Hospital and to determine factors related to these infections. **Methodology:** Blood samples were gathered from 121 aborted women attended the hospital, from June 2016 to February 2017. Samples were examined for specific IgM and IgG antibodies against *Toxoplasma*, *Rubella*, and *CMV* by Chemiluminescent Microparticle Immunoassay (CMIA) technology. **Results:** About two thirds (64.5%) of participants were positive for at least one of the studied infections. The specific IgM antibodies were positive in 1 case for *Toxoplasma*, 3 cases for *Rubella*, and 5 cases for *CMV*. Specific IgG antibodies were positive in 45 cases for *Toxoplasma*, 33 cases for *Rubella*, and 48 cases for *CMV*. Multiple logistic regression analysis identified that rural residence was significantly linked to *Toxoplasma* (OR=8.35, p-value <0.001), *CMV* (OR=2.26, p-value = 0.042), *Rubella* (OR = 3.7, p-value=0.003) infections. Seropositivity for multiple infections was detected in 33.1% of participants. Rural residence and high parity were significantly connected with multiple infections (p-value <0.001 and 0.045 respectively). **Conclusion:** Infection with the studied agents is highly prevalent (64.5%) among the studied women where antibodies against *Toxoplasma*, *Rubella* virus and *CMV* were found in different proportions (38 %, 29.8 %, and 43.8 % respectively). Rural residence is a strong predictor of these infections. Hence the country's health authorities must be alerted, and preventive measures should be taken.

INTRODUCTION

Abortion is the spontaneous loss of pregnancy during the first 24 gestation weeks. It occurs in one of five pregnancies and can have enormous physiological and psychological impacts on the patient¹.

Infections risen by *Toxoplasma*, *Rubella* and *Cytomegalovirus* are principal causes of abortion among humans. Women affected with one of these morbidities during pregnancy are at elevated risk for miscarriage, stillbirth, or a child with grave birth faults².

Toxoplasmosis, caused by the obligate intracellular protozoan *Toxoplasma gondii*. It is a critical zoonosis with medical and veterinary hazards worldwide. The disease is mainly initiated by ingesting undercooked or raw meat including viable tissue cysts, or by ingesting water or food polluted with oocysts³. Maternal infection with *toxoplasmosis* throughout pregnancy is extremely related to transplacental transmission⁴.

Rubella is termed German measles. It was among the major serious childhood diseases, which occurred as a postnatal infection⁵. *Rubella* infections result in relatively minor illness in most patients, including a

maculopapular rash in many cases, which may sometimes get misdiagnosed as measles⁶. During pregnancy, clinical diagnosis of *Rubella* is complicated as only 50% of the infected people exhibit with typical exanthematous skin lesions⁷.

The human *Cytomegalovirus (CMV)* is among the gigantic elements of congenital infections. Its clinical presentation extent from asymptomatic sorts (90% of cases) to intense fetal damage and, in rare cases, abortion and death⁸. *Cytomegalovirus* infection throughout pregnancy is extremely complicated than other infections, fetal infection varies from 33% to 75% as gestation progresses, and occurrence of disease may reach up to 50% if infection subsists throughout the first half of pregnancy⁹.

This study aims at affording screen study about abortion in Sohag, Egypt with detection of *Toxoplasma*, *Rubella* and *CMV* infections among aborted women attending Sohag University Hospital and determining factors related to these infections.

METHODOLOGY

Study design and population:

This study was carried out in the Clinical Pathology Department, Faculty of Medicine, Sohag University Hospital, Sohag, Egypt.

This cross-sectional study was carried out from June 2016 to February 2017. A total of 121 women with complete first trimester abortion attended the Outpatient Clinic were registered in the study after securing their informed consent. Their ages ranged from 18-38 years. Exclusion criteria included other causes of fetal loss diagnosed by clinical inspections and laboratory investigations, e.g. diabetes mellitus, hypertension, Rh incompatibility, and consanguinity. Data about age, residence, and parity were collected.

Ethical consideration:

Before starting data collection, ethical approval was secured from the Scientific Research Ethical Committee of Faculty of Medicine, Sohag University. During data collecting step, an informed agreement was secured from each participant.

Blood collection and serological tests

Five ml of venous blood were withdrawn from each patient by venipuncture in plain tubes for serological testing. Samples were screened for detection of IgM and IgG antibodies against *Toxoplasma*, Rubella and Cytomegalovirus by ARCHITECT i2000SR system using ARCHITECT IgM and IgG antibodies supplied by Abbott. IgM and IgG antibodies were estimated by a Chemiluminescent Microparticle Immunoassay (CMIA) technology.

Results were expressed qualitatively as positive and negative. In event of equivocal outcome, a second specimen was obtained in 7 to 14 days and if still equivocal, it was excluded from data analysis. Cut-off points calculations were done according to manufacturers' recommendations to categorize seropositive samples.

Statistical analysis

The statistical analysis was applied utilizing The IBM SPSS Statistics for Windows version 22. Sample characteristics were summarized as numbers and percentages for categorical variables and quantitative data was expressed as means \pm standard deviation, median, and range. Shapiro-Wilk test was applied for testing data normality. Mann-Whitney U test was applied for data which wasn't normally distributed. Chi-Square test was applied for comparison between qualitative variables. The associations between the studied determinants and seropositivity for any of the studied infections and multiple infections were examined at both univariate and multivariable levels using binary logistic regression. A 5% level was chosen as a grade of statistical significance in all statistical tests used in the study.

RESULTS

This study involved 121 women (64 urban & 57 Rural) attended the Outpatient Clinic of the Clinical Pathology Department, Faculty of Medicine, Sohag University Hospital. Their age ranged from 18 to 38 years, with a mean \pm SD value of (26.6 \pm 4.9). Primigravida women represented 47.9% compared to 52.1 % who were multigravida. (Table 1) As illustrated by figure (1), about two thirds (64.5%) of the participated women were positive for at the minimum one of the specified infections, while 35.5% of the tested samples were negative for all the studied organisms.

Table 1: Characteristics of the participated women

Characteristics	Summary statistics
Age(years)	
Mean \pm S.D.	26.6 \pm 4.9
Median (Range)	26 (18- 38)
Residence	
Urban (%)	64 (52.9%)
Rural (%)	57 (47.1%)
Parity	
Primigravida (%)	58 (47.9%)
Multigravida (%)	63 (52.1%)

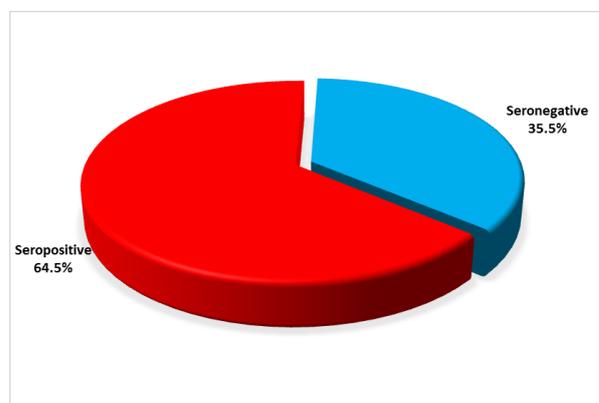


Fig. 1: Seropositivity against any of the studied infections

Regarding *Toxoplasma* infection, 37.2% of the examined samples were positive for IgG *Toxoplasma* and 0.8% for IgM. More than one third (39.7%) were positive for CMV IgG and 4.1% for CMV IgM. For Rubella virus, 27.3% were IgG positive and 2.5% were IgM positive (Table 2).

Table 2: Seropositivity of IgG and IgM antibodies against Toxoplasma, CMV and Rubella infection among the studied women.

Immunoglobulin	Toxoplasma No. (%)	CMV No.(%)	Rubella No.(%)
IgG	45 (37.2%)	48(39.7%)	33(27.3%)
IgM	1 (0.8%)	5(4.1%)	3 (2.5%)

Multiple logistic regression analysis exhibited that rural residence was significantly linked to Toxoplasma (OR = 8.35, P-value <0.001), CMV (OR = 2.26, P-value =0.042) and Rubella (OR=3.7, P-value=0.003) infections (Table 3).

Table 3: Logistic regression analysis of factors associated with Toxoplasma, CMV and Rubella infection.

Characteristics	OR (CI 95%)	P- value	Adjusted OR (CI 95%)	P- value
Toxoplasma				
Age	0.98 (0.91– 1.05)	0.551	1.06 (0.96 – 1.18)	0.217
Residence	5.96 (2.63 – 13.51)	<0.001	8.35 (3.29 – 21.18)	<0.001
Parity	0.71 (0.34– 1.5)	0.361	0.34 (0.12–0.94)	0.07
CMV				
Age	1.04 (0.97– 1.12)	0.268	1.03 (0.94– 1.13)	0.524
Residence	2.28 (1.09 – 4.78)	0.029	2.26 (1.03– 4.96)	0.042
Parity	2.15 (1.02– 4.53)	0.043	1.69 (0.69– 4.14)	0.25
Rubella				
Age	1.02 (0.94 – 1.1)	0.615	1.02 (0.92– 1.13)	0.712
Residence	3.76 (1.64– 8.67)	0.002	3.7 (1.54– 8.88)	0.003
Parity	1.99 (0.89– 4.44)	0.093	1.57 (0.58– 4.28)	0.38

Antibodies against multiple infections were discovered in 40 (33.1%) participants where 14.9 % tested positive for both CMV IgG and Rubella IgG, 7.5 % were positive for Toxoplasma IgG, CMV IgG and Rubella IgG, 5.8% were positive for Toxoplasma IgG

and CMV IgG, and 1.7% were positive for Toxoplasma IgG and Rubella IgG. Out of the participated 121 women, 4 cases were positive for IgM and IgG for multiple infections (Table 4).

Table 4: Seropositivity of IgG and IgM antibodies against multiple infections (N.=40).

Immunoglobulin	No. (%)
IgG only	
CMV+ Rubella	18 (14.9 %)
Toxoplasma+ CMV+ Rubella	9 (7.5%)
Toxoplasma+ CMV	7 (5.8%)
Toxoplasma+ Rubella	2 (1.7%)
Both IgM and IgG	
Toxoplasma IgG+ Rubella IgM	1 (0.8%)
Toxoplasma IgG+ CMV IgM+ Rubella IgM	1 (0.8%)
Toxoplasma IgG+ CMV IgG+ CMV IgM+ Rubella IgM	1 (0.8%)
Toxoplasma IgG+ CMV IgG+ CMV IgM+ Rubella IgG	1 (0.8%)

Rural residence and high parity were significantly connected with multiple infections where the proportion of the participants with multiple infections was significantly higher in those who resided in rural areas than urban residents (49.1% vs 18.8%, P-value < 0.001)

and among multigravida than primigravida participants (41.3% vs 24.1%, P-value= 0.045). On contrary, age was not significantly linked to the emergence of multiple infections (Table 5).

Table 5: Relation between seropositivity for multiple infections and sociodemographic variables

Characteristics	Yes N.=40 (33.1%)	No N.=81 (66.9%)	P-value
Age(years) Mean± S.D. Median (Range)	26.9 ± 4.7 25.5 (18- 36)	26.6 ± 5.1 26 (18- 38)	0 .960*
Residence Urban (%) Rural (%)	12 (18.8%) 28 (49.1%)	52 (81.2%) 29 (50.9%)	<0.001**
Parity Primigravida (%) Multigravida (%)	14 (24.1%) 26 (41.3%)	44 (75.9%) 37 (58.7%)	0 .045**

*P-value was calculated by Mann Whitney U test

**P-value was calculated by Chi-square test

Furthermore, on univariate regression analysis, residence (OR = 4.18, P-value = 0 .001) and high parity (OR = 2.21, P-value = 0.047) were significantly linked to multiple infections among the study population.

Multiple regression analysis identified residence only as an independent indicator of infection with more than one of the studied organisms (OR = 3.75, P-value = 0.002) (Table 6).

Table 6: Logistic regression analysis of factors associated with multiple infections.

Characteristics	OR (CI _{95%})	P - value	Adjusted OR (CI _{95%})	P - value
Age	0.99 (0.93– 1.08)	0 .975	0.98 (0 .88– 1.08)	0.658
Residence	4.18 (1.85– 9.45)	0 .001	3.75 (1.61– 8.75)	0.002
Parity	2.21 (1.01– 4.83)	0 .047	2.21 (0 .81– 6.04)	0.121

DISCUSSION

There is a considerable variation in the development of *Toxoplasma gondii*, Rubella virus, and CMV infections among women in the childbearing age in different geographical areas, socioeconomic status, races, and age¹⁰ and this might be due to variations in the immunization rates¹¹.

Serologic testing is among the fundamentals of diagnosis including the titration of serum for IgG, IgM antibodies. IgG antibodies are very helpful for screening intents and detecting continuation of active infection¹². IgM antibodies are specific; however, their presence may hold for several months after initial infection¹³.

In our research, we utilize CMIA technology for detection of IgM and IgG antibodies against *Toxoplasma*, Rubella, and CMV.

The present study exhibited that 37.2% of the participants were positive for IgG *Toxoplasma* and 0.8% for IgM, 39.7% were positive for CMV IgG and 4.1% for IgM and 27.3% were positive for Rubella IgG and 2.5% for IgM.

In Libya, Saad and Yousef¹⁴, revealed that the overall prevalence of *Toxoplasma*, Rubella and CMV IgG, and IgM antibodies in pregnant women who experienced abortion in recent pregnancy, was high for Rubella IgG (98.59%), followed by CMV IgG

(95.77%), and *Toxoplasma* IgG (39.43%), while IgM anti-Rubella, anti-*Toxoplasma*, and anti-CMV were (0%), (0%) and (2.81%) respectively, this difference may be explained by smaller sample size of the later study (70 cases).

In Turkey, Sirin et al.¹⁰ have also reported overall *Toxoplasma*, Rubella, and CMV IgM and IgG antibody positivity. The rates of IgG positivity for *Toxoplasma* was 32.3%, Rubella virus was 93.5%, and CMV was 98.9%. Immunoglobulin M antibodies were positive in 1.9% for *Toxoplasma*, 1.2% for Rubella, and 1.5% for CMV.

Another study was carried out in Brazil and revealed that 62% of the pregnant women were positive for *Toxoplasma* IgG, 3.4% were positive for *Toxoplasma* IgM, 93.1% were positive for Rubella IgG and only 0.6% tested positive for Rubella IgM¹⁵.

Rural residence was significantly linked to *Toxoplasma*, CMV and Rubella infections and this may be due to the existence of domestic animals and the favorable environmental factors, while age and parity were not significantly connected to any of the specified infections which is consistent with the results of Tammam et al.¹⁶, who identified rural residence as a significant indicator for *Toxoplasma* infection while age and parity had no role in the occurrence of the infection.

Furthermore, Kamal et al.¹⁷ found that rural residence was significantly related to toxoplasma infection.

In contrast, Negero et al.¹⁸, found that parity was among the significant predictors of Toxoplasma infection, but residence had no role and Endris et al.¹⁹, observed that no significant relationship between residence and Toxoplasma infection. Additionally, another study performed by Maingi and Nyamache²⁰, found that parity was among the significant indicators of CMV infection while residence wasn't.

In agreement with the present study, Ogbaini-Emovon et al.²¹, observed the insignificant relationship between CMV infection and participants' age and parity.

The present study exhibited that antibodies against mixed infections were detected in 33.1% of participants where 14.9% were positive for both CMV IgG, and Rubella IgG, 7.5 % were positive for Toxoplasma IgG, CMV IgG and Rubella IgG, 5.8% were positive for Toxoplasma IgG and CMV IgG and 1.7% were positive for Toxoplasma IgG and Rubella IgG. Out of the participated 121 women, 4 cases were positive for IgM and IgG for multiple infections Shrivastava et al.²² detected IgG for mixed Toxoplasma, Rubella and CMV in 3.12 % of respondents, IgM for Toxoplasma, Rubella and CMV in 3.12%, IgG for CMV and Rubella in 32.8%, IgG for Toxoplasma and CMV in 3.12%, IgM for Toxoplasma and CMV in 1.6% and IgM for Rubella and Toxoplasma in 7.8%.

Higher levels of mixed infections were detected by Saad and Yousef¹⁴ where IgG for Rubella and CMV were found together in 94% of the respondents. Furthermore, Hamdan et al.²³, found that 46.75% of participants had IgG for CMV and Rubella.

Rural residence and high parity were significantly related to multiple infections. On contrary, age was not significantly linked to the occurrence of multiple infections. Multiple regression analysis identified residence only as an independent indicator of multiple infections.

In the study performed by Hamdan et al.²³, univariate and multivariate analysis clarified that high parity was a significant indicator of CMV infection. Age was significantly associated with CMV infection in univariate analyses. Additionally, the finding of Andiappan et al.²⁴, revealed that age was among the significant factors related to *Toxoplasma* infection.

CONCLUSION

Infection with the studied agents is highly prevalent (64.5%) among the studied aborted women where antibodies against Toxoplasma, Rubella virus, and CMV were detected with different proportions (38 %, 29.8 %, and 43.8 % respectively). Rural residence was demonstrated to be an important predictor of these infections. Furthermore, rural residence was

significantly related to multiple infections. Therefore, it is recommended that all antenatal cases with such history should be subjected to routine screening for these pathogens. Early diagnosis will help in the management of the cases. This study also highlighted the need for immunization of prospective mothers and adolescent girls who had not received the vaccine in their childhood which provides the needed immunity to hinder infection and accordingly reduce the chance of abortion.

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