Original Article

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Seroepidemiology of *Toxoplasma Gondii* Infection in Women with First Trimester Spontaneous Miscarriage in Qena Governorate, Egypt

Obstetrics and Gynaecology Section

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ABSTRACT

Introduction: To determine seroprevalence and risks factors for *T. gondii* in women with early miscarriage, Sera of 76 women were analyzed infection by indirect enzyme linked immunosorbent assay (ELISA). Seropositive cases were further examined histopathologically for evidence of *Toxoplasma gondii* organisms.

Material and Methods: Demographic data were obtained from participants to gather information on risk factors.

Result and Discussion: Of 76 women with spontaneous abortion screened for Toxoplasma-specific IgG and IgM antibodies with ELISA, 35 were IgG seropositive, of which, 14 samples were IgM seropositive. Therefore, seropositivity rates of 46.1% (95% CI: 35.1%, 57.3%), and 18.4% (95% CI: 10.89%, 28.32%) for IgG and IgM, respectively were found. These indicate that, 27.6 % (21 cases) of studied women (IgG+/IgM-) were immune to toxoplasmosis and 53.94 %(41 cases) were susceptible to primary infection (IgG-/IgM-). Mean while acute toxoplasmosis (IgG+/IgM+) was 18.4 %(14 cases) with one case (1.3%) confirmed for recent infection as she had Tachyzoites on histopathology study. On the basis of multivariate logistic regression, living in a rural area was found to be the only independent predictor of toxoplasmosis (OR=3.800, CI= 1.100-10.813, p=0.034).

Conclusion: The seroprevalence of *T. gondii* infection in women with first trimester abortion in Qena governorate of Egypt is high. Pregnant women living in rural area are at a higher risk for acquiring infection during pregnancy. Antenatal screening of pregnant women and educational program about risks for Toxoplasmosis in rural areas is needed.

Keywords: Toxoplasmosis, Abortion, Immune response, Epidemiology, Pregnancy, Egypt

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan organism occurring in domestic animals and man throughout the world. Human acquired infection by ingestion of undercooked meat infected with oocystes. The clinical illness seldom results but, congenital infection as a cause of reproductive disorders has been recognized for some time [1]. In women, congenital infection can lead to congenital toxoplasmosis especially in immune-compromised subject [1]. However, congenital infection can leads to severe disease when the infection is acquired in the first trimester [2]. Congenital infection can lead to a wide range of manifestations in the fetus including spontaneous miscarriage or still-birth. Complications In a living infant with congenital toxoplasmosis include microcephalus or hydrocephalus, retinochorioditis and cerebral calcifications, failure to thrive or an apparently normal infant who develops symptoms of CNS involvement later in life [3].

It has been estimated that one third of the world population has been infected with toxoplasmosis [4,5]. According to a World Bank report published in 2012, rural population in Egypt was last reported at 57.2%, most of them are working on farms with an increasing number of poor people. A previous study has shown a 57.9% seroprevalence rate of *T. gondii* among pregnant women in Egypt [6]. This high seroprevalence rate of *T. gondii* may be responsible for a significant number of early miscarriages among this population. The aim of the current study was to determine the seroprevalence and risks factors for toxoplasmosis among women with first trimester miscarriage.

SUBJECT AND METHODS

In this prospective cross-sectional hospital based-study, 76 women with first trimester miscarriage underwent evacuation at Qena University Hospital South Valley University, Egypt from January, 2012 through March, 2013. This study was approved by the Ethics Committee of Qena University Hospital South Valley University, and a written consent was obtained from each participant.

Inclusion Criteria

Women with spontaneous miscarriage at 2-8 weeks gestations. Gestational age was determined by the last menstrual period and was confirmed by ultrasound scan.

Demographic data, including maternal age, parity, duration of marriage and educational level were recorded. Outcomes were assessed included antibody titers for Both IgG/IgM, and the histopathology of the conceptus materials for those with positive titer.

Technique: From all subjects blood was drawn for serological testing. All conceptus materials, of seropositive persons were further processed for Histopathological study.

Serological Detection of *Toxoplasma gondii* infection

Five ml of venous blood were collected aseptically from each of the study participants. Then serum was separated from the whole blood by centrifugation at 3000 rpm for 5 minute. Sera were isolated and kept in sterile microtubes at -20ºC until use for serological examinations. All the collected specimens were tested for IgM and IgG anti-*Toxoplasma gondii* antibodies by enzyme-linked immunosorbent assay (ELISA) test kit (SeraQuest® TOXOPLASMA) provided from Quest International, Inc. Miami, FL, USA); following the manufacturer's instruction.

Parasitological Detection of *Toxoplasma gondii* Infection

The tissue slides were deparaffinized and rehydrated through graded alcohols to water. Then, they were rinse in pH 6.8 buffered

distilled water, stained in working Giemsa, overnight and rinsed and dehydrated gradually and cleared, mounted and covered by cover slide.

Histopathology

Samples of miscarriage materials were collected and immersed into 10% neutral buffered formalin for fixation. Samples were dehydrated and embedded in paraffin wax in the usual manner, sectioned (4 µm thick) and stained with hematoxylin and eosin (H&E). Two to three paraffin embedded blocks were prepared for each specimen. Tissues were investigated for toxoplsama schizont, male and female gametes.

STATISTICAL ANALYSIS

The Statistical Package for the Social Sciences for Windows, version 15 (SPSS Inc., Chicago, Illinois) was used to record data and for analyses. The descriptive analyses used included the mean, standard deviation, and frequency distribution. For logistic regression using multivariate analysis, independent variables were added to the model at the same time, whereas in the univariate analysis, each variable was entered separately. The results of the analysis are presented as odds ratios (ORs) and 95% confidence intervals (95% CIs). A p value <0.05 was considered significant.

RESULTS

The total number of cases in this study was 76 patients with first trimester miscarriages (2 to 8 weeks gestations). The study revealed that *T. gondii* IgG antibodies were detected in 46.1% (n=35) of the samples; of which 27.6% were positive for IgG and 18.4% positive for both IgG and IgM antibodies. Therefore, seropositivity rates of 46.1 % (95% CI: 35.1%, 57.3%), and 18.4% (95% CI: 10.89%, 28.32%) for IgG and IgM, respectively were found. The mean IgG and IgM levels were (16.7714±5.39109 and 8.5714±11.01946) respectively.

The mean maternal ages of the studied women were 26.8158 ± 5.474±409 years, ranged (19 to 36) years. Their mean parities were 2.3684±1.67206, ranged (0 to 5) deliveries.

[Table/Fig-1] shows the selected socio-demographic characteristics of the studied population and the association with serology for toxoplasmosis. The seroprevalence of toxoplasmosis was found to be higher among women less than 25 years of age (42.1%) and lowest among 25-29-year-old (18.4%). Again, the seroprevalence of toxoplasmosis was more among women with secondary education (31.4%), more often multiparous (65.7%) and living in rural areas (65.7%). It was less common among 25-29-year-old women (18.4%) and those with basic education (20.0 %) and those living in urban areas (31%).

The histological findings in the majority of the conceptus materials of seropositive women revealed areas of focal necrosis with leukocytic infiltrations of the deciduas. Furthermore, deciduas infiltrations with mononuclear cell with vasculitis and perivascular edema were reported in some cases [Table/Fig-2].

As regard to the parasitological examination of the conceptus material, the tachyzoites and the Toxoplasma cyst were demonstrated in one case [Table/Fig-3].

In [Table/Fig-4], univariate and multivariate analysis of sociodemographic characteristics of the studied women showed that there was a significant association between seroprevalence of toxoplasmosis and living in rural areas.

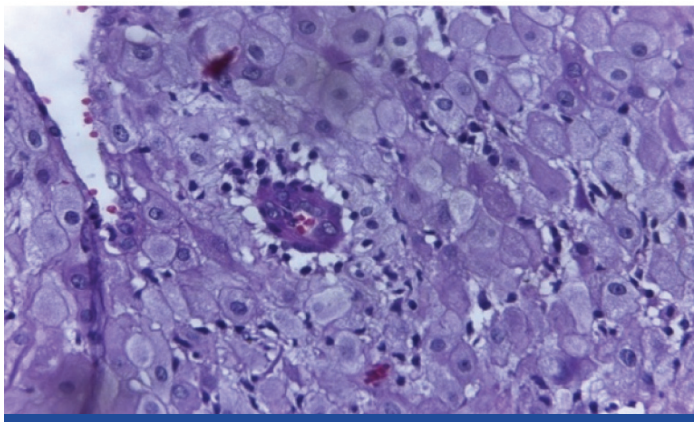
DISCUSSION

The diagnosis of toxoplasmosis poses a challenge for health care givers due to the complexity in the interpretation of the results. The diagnosis of toxoplasmosis in human is common done by serologic tests (in which the interpretation should be done meticulously), PCR and histologic demonstration of the parasite [7]. However, biopsy should be used in selective cases because it is an invasive.

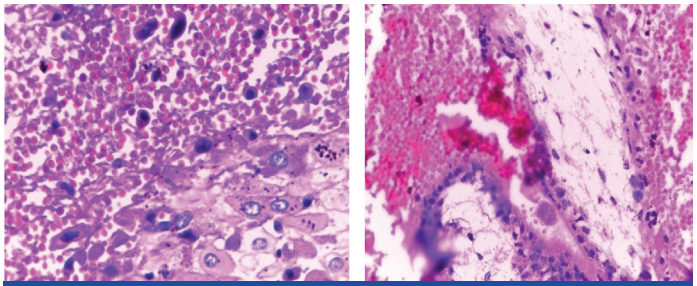
In the current study, the seroprevalence of IgG antibodies to *T. gondii* in women with early spontaneous miscarriage was found to be 46.1%. A similar previous study from Qualyobia governorate reported a seroprevalence of 44.7% among aborted women, which

Characteristics	Frequency (%)	Serology	
Age group (years)		**Negative**	**Positive**
<25	32(24.8)	16(39.0)	16(42.1)
25-29	14(10.9)	8(19.5)	6(18.4)
30 and more	30(23.3)	17(41.5)	13(39.5)
Educational level			
Illiterate	20(15.5)	11(26.8)	9(25.7)
Basic	18(14.0)	11(26.8)	7(20.)
Secondary	20(15.5)	9(22.0)	11(31.4)
University	18(14.0)	10(24.4)	8(22.9)
Total number of pregnancies			
Primi	16(12.4)	8(19.5)	8(22.9)
Multi	49(38.0)	26(63.4)	23(65.7)
Grand	11(8.5)	7(17.1)	4(11.4)
Residence			
Urban	36(27.9)	24(58.5)	12(34.3)
Rural	40(31.0)	17(41.5)	23(65.7)
Serology of toxoplasma			
Negative	41(31.8)		
Positive	35(27.1)		

[Table/Fig-1]: Socio-demographic characteristics of the studied women and the association with the prevalence of toxoplasmosis
Values are presented as number (percentage)



[Table/Fig-2]: The decidua shows a mononuclear cell infiltration with vasculitis and perivascular edema from conceptus material at evacuation in a-33-year-old woman with first trimester abortion complicated with toxoplasmosis (H&E x100)



[Table/Fig-3]: Banana-tachyzoites: obtained from conceptus material at evacuation in a33-year-old women with first trimester abortion complicated with toxoplasmosis (H&E x100)

Toxoplasma gondii cysts obtained from conceptus material at evacuation in a33-year-old woman with first trimester abortion complicated with toxoplasmosis (H&E x100)

Variable	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Age group						
<25	1.00*					
25-29	1.308	0.481-3.558	0.599	0.982	0.045-1.723	0.169
30 and more	0.981	0.272-3.532	0.976	0.596	0.027-1.892	0.170
Parity						
Primigravida	1.00*					
multigravida	1.750	0.364-8.424	0.485	0.991	0.635-410.730	0.092
grand multiparas	1.548	0.401-5.975	0.526	0.673	0.779-114.403	0.078
Residence						
urban	1.00*					
rural	2.706	1.063-6.889	0.037	3.800	1.100-10.813	0.034
Educational level						
University	1.00*					
Illiterate	1.023	0.284-3.681	0.973	0.637	0.147-2.765	0.547
Basic	0.795	0.211-3.000	0.735	0.906	0.205-4.009	0.896
secondary	1.528	0.424-5.499	0.517	1.963	0.454-8.480	0.366

[Table/Fig-4]: Logistic regression analysis of predictors of T. gondii infection in women with first trimester spontaneous miscarriage
Abbreviations: OR, odds ratio; CI, confident interval; p-value was set significant at <0.05
1.00* reference category

comparable with our findings [6]. However, the seroprevalence found in women with early miscarriage is lower than seropositivity reported recently in other governorates in the country, Menoufia [8] in 67.5% and Gharbia [9] in 52.4% of cases, indicating that infection with toxoplasmosis is on the rise. The 46.1% seropositivity rate is high when compared to Dakahlia (23.85%) [10], and EL Fayoum (30.5%) [11] [Table/Fig-5]. As regards to the nearby countries; the seroprevalence in the current study is comparable to Tunisia [12] in 39.3%, but it is higher compared to seroprevalence of toxoplasmosis in Sudan [13] in 34.1% and Palestine [14] in (27.9%) of cases. However, it is much higher than the 31·0% found in younger women in their reproductive age in Italy [15], and the 15.0% found in women aged 15-44 years in United states [16].

The variations in the seroprevalence rate whether nationally or in nearby countries should be interpreted cautiously since different tests with different sensitivity and specificity were used, along with different population having different susceptibility to infection as evident in [Table/Fig-5]. Moreover, the seroprevalence may be influenced by the study population, age, sample size, study area, number of cat and their infectivity and geographical variation may account for some of the differences in the reported seroprevalence [4, 17].

In the current study, the seroprevalence of IgM for toxoplasmosis was found be 18.4 % (n=14). It is comparable with most of the governorates in Egypt except for Qualyobia and El Fayoum that reported higher seroprevalence for IgM [Table/Fig-5]. High environmental contamination (97.4%) with *T. gondii* oocytes from rats [18], in addition to inadequate hygiene, and suitable climatic factors for survival of oocysts might be responsible for this high seropositivity.

In the current study, there were 21(27.7%) patients with (IgG+/IgM-) results, which indicates infection with the organism at some time, usually old infection, while there were 14(18.4%) patients with (IgG+/IgM+) results, which indicates recent infections. We cannot grantee that it was responsible for pregnancy loss in this study. In pregnancy, it is mandatory to perform additional conclusive tests that must include, IgG avidity test, PCR, IgA and IgE, on individuals with both positive IgG and IgM [19], because Toxoplasma-specific IgM antibodies may be persist as long as 18 months after acute acquired infection [20].

T. gondii infection was 3.8 times greater in individuals who live in rural areas than those in urban areas. Our results were in agreement with El-Gozamy et al., study which was conducted between August 2007 and October 2008 in Egypt, concluded that seropositivity for *T. gondii* among pregnant women was relatively high in the rural (57.6%) than urban (46.5%) areas [12]. High positivity for toxoplasmosis in rural areas may indicates the life style of the inhabitants which makes them more prone to the infection, again high density of domestic animals in rural areas as well as the favorable environmental conditions for *T. gondii* oocysts to sporulate. Unwashed food, lack of sanitary water may all contribute to high positivity in rural areas [21]. In the current

	Seroprevalence of *T. gondii*										
	In governorates of Egypt					In other countries					
Variables	Qualyobia	El Fayoum	Menoufia	Dakahlia	Gharbia	Sudan (Khartoum)	Tunisia (Tunisia)	Palestine Hebron	Palestine	Central Italy	United States
Year of study	2001	2006	2012	1993	1997	2003	1994-2006	2005	2005	2012	1988-1994
population	Aborted women	Pregnant and non pregnant	Pregnant women	Different localities	Slaughter	Pregnant women	Population	Rural	Urban	Younger women	Women 15-44 years
Sample size	38	88	323	320	162	487	40,566	2005	2005	13 000	17,658
Test used	ELISA	ELISA	ELISA	Dot-ELISA	IHAT	ELISA	ELISA	ELISA	ELISA	ELISA	ELISA
IgG %	44.7%	30.5%	67.5%	23.8%	52.4%	34.1%	47.7%	21.4%	21.4%	31·0%	15.0%
IgM %	23.7%	24.2%	2.8%	-	-	14.3%	-	-	-	1·6%	-

[Table/Fig-5]: Comparison of sero prevalence of T. gondii in different Governorates of Egypt and some selected countries
ELISA, indirect enzyme linked immunosorbent assay; IHAT, indirect hemoagglutination test

study, there was no association between an increasing maternal age and seropositivity for *T. gondii* as reported by others authors [22,23].

In this study, tachyzoites were isolated from the conceptus materials in one case (2.9%) of the 35 seropositive cases. Biopsy is considered the most definitive method of diagnosis for toxoplasmosis because this method may demonstrate the presence of tachyzoites. It has been reported that toxoplasmosis has a significant relation with first trimester miscarriage [24]. The mechanism by which toxoplasmosis induced abortion is either inhibits or triggers the apoptosis with excessive levels of Th1 cytokines, particularly IL-18 and IFN- γ (Nishikawa et al., 2002) [25]. This indicates that toxoplasmosis induce abortion through a chain of immunological reactions, therefore negative biopsy does exclude *toxoplasma gondii* as a causative agent.

CONCLUSION

In the current study high seropositivity for toxopalsma gondii was reported (18.4%) indicating potential for abortion and congenital transmission. Women living in rural areas are at higher risks for

T. gondii infection. Antenatal screening of pregnant women and educational program about risk for Toxoplasmosis in rural areas is needed.

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