



Seropositivity of Toxoplasmosis in Pregnant Women by ELISA at Minia University Hospital, Egypt

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Abstract: Toxoplasmosis is considered as an important risk factor for bad obstetric history (BOH) and one of the major causes of congenitally acquired infections. The present study aimed to estimate the seropositivity of *T. gondii* infection and associated risk factors among the attendees of high risk pregnancy and low risk antenatal care clinic of Minia Maternity and Pediatric University Hospital, Minia, Egypt. The study was carried out from April 2013 to April 2014 through 2 phases, the first phase was case-control study, and the second phase was follow-up with intervention. A total of 120 high risk pregnant and 120 normal pregnant females were submitted to clinical examinations, serological screening for anti-*Toxoplasma* IgM and IgG antibodies by ELISA, and an interview questionnaire. Seropositive cases were subjected to spiramycin course treatment. The results showed that the seroprevalence of toxoplasmosis in high-risk pregnancy group was 50.8%, which was significantly different from that of normal pregnancy group ($P < 0.05$). Analysis of seropositive women in relation to BOH showed that abortion was the commonest form of the pregnancy wastage (56.5%). The high prevalence of *T. gondii* seropositive cases was observed in the age group of 21-30 years. Post-delivery adverse outcome was observed in 80.3% of high-risk pregnancy group compared to 20% of normal pregnancy group. There was a statistically significant relationship between seropositivity and living in rural area, low socioeconomic level, and undercooked meat consumption ($P < 0.05$). Serological screening for anti-*Toxoplasma* antibodies should be routine tests especially among high-risk pregnant women.

Key words: *Toxoplasma gondii*, toxoplasmosis, high-risk pregnancy, neonatal outcome, seropositivity, ELISA

INTRODUCTION

Toxoplasma gondii is one of the opportunistic obligate intracellular parasites with cats as the definitive host, while humans and animals act as intermediate hosts [1]. Human infections occur by different ways such as ingestion or handling of raw or partly cooked meat containing tissue cysts, direct contact with cats, and/or from the consumption of water or food contaminated by oocysts discharged in the feces of infected cats [2].

Congenital toxoplasmosis occurs when mother is primarily infected with *T. gondii* during pregnancy, and the infection spreads to the developing fetus across the placenta with possible serious problems for the fetus, including chorioretinitis, intracranial calcifications, hydrocephalus, and even stillbirth

[3]. The degree of severity of the disease depends on the gestational age as severe fetal affection occurred with early gestational age infection [4].

Toxoplasmosis is considered as an important risk factor for bad obstetric outcome (BOH) and one of the major causes of congenitally acquired infections [5,6]. Therefore, early diagnosis of toxoplasmosis is a crucial step to start treatment on time to minimize the trans-placental transmission [4,7]. Toxoplasmosis is usually diagnosed by serological tests that measure *Toxoplasma* specific IgM and IgG antibodies [3]. The prevalence of chronic *Toxoplasma* infection by serological test was 64.7% in Egypt [8].

Thus, this study aimed to determine the extent of seropositivity of *T. gondii* infection among antenatal women with BOH and previous normal obstetric history attending antenatal care clinic of Minia Maternity and Pediatric University Hospital, Minia, Egypt. In addition to analyze the seropositivity in relation to the type of BOH and the age of the study subjects, the current study conducted to assess the outcome of current pregnancy among those with seropositivity. Also, the research

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aimed to correlate the seropositive patients to the demographic risk factors of *T. gondii* among the study groups.

MATERIALS AND METHODS

Study design and setting

This study was carried out between the period of April 2013 to April 2014 at the high risk pregnancy care clinic and the low risk antenatal care clinic of Minia Maternity and Pediatric University Hospital, Minia, Egypt.

Inclusion criteria of antenatal women of reproductive age with previous BOH (group I) was spontaneous abortion, missed abortion, intrauterine fetal death, still births, preterm deliveries, congenital malformations, perinatal death, unexplained early neonatal death, women having mentally retarded children in earlier pregnancies, and unexplained seizures. Exclusion criteria by clinical examination and laboratory investigations was other causes of fetal wastage, such as hypertension, diabetes mellitus, Rh incompatibility, physical causes of abortion, and consanguinity. Subjects with known causes of fetal wastage were excluded from the study. Normal pregnancy (group II) included multiparous age-matched antenatal women without BOH attending the same clinic.

All seropositive cases were treated with spiramycin in a dose of 3 million IU twice a day and for 2 weeks (according to the standard protocol of the Department of Obstetrics and Gynecology, Faculty of Medicine, Minia University). Then, they were followed to assess the outcome of their pregnancy.

To work out the required sample-size for the determination of seropositivity in antenatal women with BOH, the equation suggested by Schiesselman [9] was used. Considering 46.2% seropositivity of toxoplasmosis in women with BOH [10] and 12.3% in women without BOH [4], the desired sample-size was 100 (50 cases and 50 controls). To increase the strength and consistency of the association the sample was increased, and the study was conducted among 120 cases and 120 controls.

Data collection

A structured questionnaire was used to investigate known toxoplasmosis risk factors (name, age, residential area, level of education, monthly income, contact with cats, precaution during cat feces handling, hand washing after handling raw meat, consumption of raw or undercooked meat, consumption of raw vegetables, and exposure to soil). The questionnaires were applied at face-to-face interviews by the researchers. The ques-

tions were answered orally by the participants and recorded by the researchers. The participant's name was written on the questionnaire, so that the risk of interviewing the same patient was eliminated.

Serology

From each of the study participants, a written consent was obtained to collect 2 ml of venous blood using sterile disposable syringes under aseptic precautions. The blood samples were then transported to the Laboratory of Parasitology Department, Faculty of Medicine, Minia University, Egypt. Serum was separated from whole blood by centrifugation at 3,000 rpm for 5 min, labeled, and kept in sterile microtubes at -20°C for further serological examinations. Each serum sample was tested for the presence of anti-*Toxoplasma* antibodies IgM and IgG using commercial ELISA kit (Cal Biotech Inc., Spring Valley, California, USA) following the manufacturer's instructions. All positive cases for anti-*Toxoplasma* antibodies were re-tested at 3 weeks interval. The results were read by optical density at 450 nm on an ELISA reader (SLT Lab Instruments, A-5082 Grödig/Salzburg, Austria, serial no. 216692). Cut-off points and antibody index calculations were done according to manufacturers' recommendations to categorize seropositive (antibody index > 1.1), borderline positive (antibody index 0.9-1.1), and seronegative (antibody index < 0.9).

Ethical approval

The study protocol was approved by the scientific ethical committee of the Department of Parasitology and the Department of Obstetrics and Gynecology, Faculty of Medicine, Minia University at their monthly meeting on January 2013. A written informed consent was obtained from each participant.

Statistical analysis of data

Statistical analysis was done with software Statistical Package for the Social Sciences (SPSS version 17). The chi-square and Z-test (test of proportion) which is a test used for testing the significant difference between only 2 proportions were used to test statistically significant differences. The odd ratio (OR) and its 95% confidence interval (CI) were used to estimate the strength of the association between the *T. gondii* infection and the associated risk factors. Statistical significance was defined as a *P*-value of < 0.05.

RESULTS

Seroprevalence of *T. gondii* infection in high-risk pregnancy group (group I) was 61/120 women (50.8%) vs 10/120 women (8.3%) in normal pregnancy group (group II). Seropositivity of both groups for *T. gondii* IgM and/or IgG antibodies was shown in Table 1 ($P < 0.05$). Among the seropositive antenatal women with BOH, abortion was the most common form of pregnancy wastage (39 cases; 56.5%) (Table 2). The high prevalence of *T. gondii* seropositive cases was observed in age group of 21-30 years (Table 3). All pregnant women in this study were followed-up until delivery. Post-delivery adverse outcomes were observed in 49 cases (80.3%) and 2 cases (20%)

in BOH and normal pregnancy, respectively (Table 4). Assessment of associated demographic risk factors among the seropositive cases was performed (Table 5). There was a significant *T. gondii* seropositivity in pregnant women living in rural area, of low socioeconomic stander, and with primary educational level, had daily contact with soil and consumed undercooked meat.

DISCUSSION

The present study showed that the seroprevalence of *T. gondii* infection among the high risk pregnancy group was 50.8% vs 8.3% among the normal pregnancy group. These results

Table 1. Seropositive rate of *T. gondii* IgM and IgG antibodies with ELISA in high risk pregnant group

Pregnant women	No. of positive <i>T. gondii</i> Abs				P-value
	IgM positive (%)	IgG positive (%)	Both positive (%)	Total (%)	
Group I (120) ^a	22 (18.3) ^c	46 (38.3) ^d	7 (5.83)	61 (50.83)	0.001
Group II (120) ^b	2 (1.66)	8 (6.66)	0 (0)	10 (8.3)	

^aHigh risk pregnancy group.

^bNormal pregnancy group.

^cIgM positive (IgM+ combined IgM and IgG).

^dIgG positive (IgG+ combined IgM and IgG).

Table 2. Previous obstetric histories in seropositive pregnancy

Type of previous BOH	No. of subtotal	No. of positive <i>T. gondii</i> antibodies (%)
Early embryonic pregnancy loss ^a	40	21 (52.5)
Late abortion (12-24) weeks ^a	29	18 (62.0)
Still birth (IUFD) delivery (24-40)	24	11 (45.8)
Preterm labor	15	7 (46.6)
Un-explained neonatal death	8	3 (37.5)
Congenital anomalies	4	1 (25.0)
Total cases	120	61 (50.83)

^aAbortion (early embryonic loss+late abortion).

Table 3. Seropositive rate of *T. gondii* IgM and IgG antibodies in high risk pregnant group according to age

Age group	Group I		Group II		P-value ^a
	No. of subtotal	No. of positive <i>T. gondii</i> Abs (%)	No. of subtotal	No. of positive <i>T. gondii</i> Abs (%)	
18-20	17	5 (29.4)	15	0 (0)	0.02 ^a
21-25	41	25 (60.9)	36	4 (11.1)	0.005 ^a
26-30	22	14 (63.6)	24	3 (12.5)	0.001 ^a
31-35	27	12 (44.4)	28	2 (7.1)	0.007 ^a
36-40	10	4 (40)	12	1 (8.3)	0.03 ^a
>40	3	1 (33.3)	5	0 (0)	0.03 ^a
Total cases	120	61 (50.8)	120	10 (8.3)	

^aP-value was calculated by Z-test (test of proportion).

Table 4. Clinical obstetric outcome(s) of seropositive pregnancy in BOH and normal pregnant groups

Clinical obstetrics outcome	No. of positive <i>T. gondii</i> Abs								P-value ^a
	Group I				Group II				
	No. of subtotal	IgM (%)	IgG (%)	Both (%)	No. of subtotal	IgM (%)	IgG (%)	Both (%)	
Abortion	18	8 (44.4)	6 (33.3)	4 (22.2)	1	1 (100)	0 (0)	0 (0)	0.09
Stillbirth (IUFD)	16	3 (18.8)	12 (75.0)	1 (6.3)	0	0 (0)	0 (0)	0 (0)	0.03 ^a
Preterm labor	13	1 (7.6)	12 (92.0)	0 (0.0)	1	0 (0)	1 (100)	0 (0)	0.2
Congenital anomalies	2	1 (50.0)	0 (0.0)	1 (50.0)	0	0 (0)	0 (0)	0 (0)	0.3
Normal baby	12	2 (16.6)	9 (75.0)	1 (8.3)	8	1 (12.5)	7 (87.5)	0 (0)	0.001 ^a
Total	61				10				

P-value was calculated by comparison between IgM, IgG and both between Group I and Group II at each obstetric outcome.

^aP-value was calculated by the Fisher's exact test.

Table 5. The correlation of demographic risk factors and *T. gondii* seropositivity in pregnant women

Demographic Characterization	No. of positive <i>T. gondii</i> Abs (%)	No. of negative <i>T. gondii</i> Abs (%)	OR (95% CI)	P-value
Residence:				
Rural	42 (59.2)	73 (43.2)	1.9 (1.08-3.3)	0.02
Urban	29 (40.8)	96 (56.8)		
Education level:				
Illiterate	2 (2.8)	7 (4.1)	0.68 (0.48-0.97)	0.03
Primary	38 (53)	29 (17.2)		
Secondary	19 (26.8)	75 (44.4)		
University	12 (16.9)	58 (34.3)		
Occupation				
House wives	44 (62)	87 (51.5)	1.5 (0.87-2.7)	0.1
Others	27 (38)	82 (48.5)		
Socio-economic level				
Low	39 (54.9)	34 (20.1)	4.8 (2.6-8.8)	0.001
Middle	29 (40.8)	102 (60.4)		
High	3 (4.2)	33 (19.5)		
Contact with soil				
Yes	38 (53.5)	64 (37.9)	1.8 (1.1-3.3)	0.02
No	33 (46.5)	105 (62.1)		
Contact with cats				
Yes	10 (14.1)	11 (6.5)	2.3 (0.9-5.8)	0.06
No	61 (85.9)	158 (93.5)		
Consumption of under-cooked meat				
Yes	49 (69)	62 (36.7)	3.8 (2.1-6.9)	0.001
No	22 (31)	107 (63.3)		
Consumption of row vegetables				
Yes	71 (100)	169 (100)	-	-
No	0 (0)	0 (0)		
Total	71 (100)	169 (100)		

P-value < 0.05.

OR, odds ratio; CI, confidence interval.

were in concordance with other previous studies [4,5,11]. The high seroprevalence of toxoplasmosis among pregnant females with complicated obstetric history were observed than among normal pregnant females.

Among the high risk group, 12.5% were seropositive for IgM antibodies which indicates acute infection, 32.5% were seropositive for only IgG antibodies which indicate chronic

infection, and 5.8% were seropositive for both IgM and IgG antibodies which indicates possible recent infection in the last 12 months. Our results were in concordance with other many similar studies [4,6,11]. In the normal pregnancy group, 6.7% were seropositive for IgG antibodies and 1.7% were seropositive for IgM antibodies. These results agreed with El Deeb et al. [8] as they found IgG percentage higher than IgM among preg-

nant women in Menofia governorate, Egypt.

Abortion was the most common form of pregnancy wastage (56.5%) in our study and agreed with other reports [4,11,12]. Also, our result was similar to a previous study, a seroprevalence of 44.7% among aborted women from Qalyobia governorate, Egypt [13]. Although some reports [4,14] stated that the seropositivity of *T. gondii* infection increases with age; however, our results showed that the seropositivity was observed more in the age group 21-30 years, which represent the child bearing age of women. The higher seroprevalence among pregnant women aged 20-30 years were also observed [15-17].

The relationship between maternal infection with *T. gondii* and the post-delivery adverse pregnancy outcomes was observed in 49 cases (80.3%) and abortion was the highest risk among the adverse pregnancy outcomes. This was agreed with the meta-analysis study done by Li et al. [18]. The studies have proved that the presence of *Toxoplasma* cysts in chronically infected uteri lead to infection of the baby in the first trimester and often to recurrent miscarriages [19].

Treatment of a pregnant woman who acquired toxoplasmosis decreases the incidence of congenital infection by approximately 60% [7]. All seropositive cases were treated with spiramycin, and 20 cases (28.2%) gave birth to normal babies. Although spiramycin reduces the risk of placental transmission, it is not always effective [20]. Additional PCR tests for amniotic fluid are recommended for the diagnosis of toxoplasmosis.

Our study showed that living in rural area, low socio-economic stander, primary educational level, and daily contact with soil were significant risk factors for *T. gondii* infection. Similar results were reported in other previous studies [10,12,17]. The life style of the residents in rural areas, the presence of domestic animals as well as the favorable environmental conditions for oocysts sporulation may contribute to this significance [21]. Also, women with higher socioeconomic standers and who were highly educated adopted appropriate hygienic measures regarding food and cooking habits which tend to eradicate the sources of disease transmission [22]. Consumption of undercooked meat was also a significant risk factor. This may be attributed to the habit of eating some Egyptian food containing undercooked meat as kabbab, shawarma, Hawawshy, and luncheon.

Negative association between *Toxoplasma* seropositivity and some documented potential risk factors among seropositive cases in this study may suggest a limited role of these factors in Minia City, Egypt. Climate and cultural differences as regards

to sanitation and food habits may contribute to this variation.

CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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