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Research Article
Associated Antenatal Health Risk Factors with Incidence of Toxoplasmosis in Egyptian Pregnant Women

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Abstract

Background and Objective: The major maternal toxoplasmosis infection during pregnancy is regularly related to trans-placental transmission to the embryo and newly-borne child. This cross-section study was performed to investigate the prevalence of toxoplasmosis among pregnant women attending antennal health centers. **Materials and Methods:** The IgM antibodies against *Toxoplasma* were quantitatively determined by commercially available kits, while IgG antibodies and avidity (AV) of *Toxoplasma gondii*-specific IgG antibodies were quantitatively determined by using of conventional ELISA. **Results:** The overall seroprevalence of *T. gondii* IgM among the investigated pregnant women was much higher than toxoplasma IgG with low IgG avidity representing acute infection with possibility of risk to the mother, embryo and newly-borne child. Results also showed that highest *Toxoplasma* prevalence was among pregnant women with history of intake of immunosuppressive drugs and abortion, having cats and animals in their households and in the 1st and 2nd trimesters. **Conclusion:** The high infection prevalence of *T. gondii* among the Egyptian pregnant women in Giza governorate revealed the risk of premature termination of pregnancy due to exposure of *T. gondii* infection.

Key words: Toxoplasmosis, antenatal, IgM antibodies, socio-demographic characteristics, risk factors, seroprevalence, pregnant women

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a mandatory intracellular parasite of apicomplexan protozoa and responsible for animal and human toxoplasmosis and one of the most common chronic diseases infecting about one-third of the world's human community[1]. Toxoplasmosis infection in animal and human hosts causing abortions and stillbirths in intermediate host animals and human[2,3]. *Toxoplasma gondii* infection routes are the oocysts ingestion shed in cat feces, *T. gondii* cysts ingestion found in chronically infected food animal tissues (undercooked or raw meat) or through the vertical transmission via placenta during pregnancy. Also, some other pathways for infection may be a blood transfusion, organs transplantation and the direct contact with the *T. gondii* infective stages[4].

The primary toxoplasmosis infection in the pregnant women is often asymptomatic or has only some mild symptoms and approximately 70-90% of infants born with congenital toxoplasmosis are asymptomatic at birth. Nevertheless, an infection may show spontaneous abortion, stillbirth, prematurity or serious fetal damage[5]. Because of the risk of congenital infection and its squeal in the newborn, the diagnosis of toxoplasmosis should be done timely and correctly. It is not easy to isolate *T. gondii* parasite, so the diagnosis is usually based on serological methods, with detection of specific *Toxoplasma* IgG and IgM antibodies and for an accurate diagnosis, the IgG avidity toxoplasma is also determined[6]. The detection of IgG avidity plays an important role in the determination of the exact time of pregnant women to be infected with *T. gondii* protozoan parasite[7].

Whereas, the IgG avidity toxoplasma plays an important role in determining the moment of pregnant infection with toxoplasmosis and differentiate acute and chronic cases, so current study determined the prevalence of toxoplasmosis among pregnant women attending antennal health centers in Giza governorate, by using IgG avidity (AV) to detect the relationship of toxoplasmosis with abortion or miscarriage and also to look any risk factors related to the infection.

MATERIALS AND METHODS

Study design and area: This cross-section study was performed between November, 2016 and June, 2017. The study participants were those all pregnant women who attended antenatal services at Antenatal Health Units in Giza Governorate, Egypt during the sample collection period. Pregnant women who were critically ill, unable to communicate and those who were not willing to provide vital information and blood samples were excluded from the study.

Sample size determination and sampling technique: The statistical formula for sample size calculation was considered, the average sero-prevalence of *T. gondii* was considered as 45% from literature and so the sample size was calculated as 380 and increased it to 388. A total of 388 blood specimens from pregnant women were collected as follows: 158 women in the first trimester of pregnancy with history of normal delivery and abortion were 111 and 47, respectively, 121 in the second and 109 in the third trimester. The age of the study participants ranged from 18-44 years.

Data collection: Information on socio-demographic data, history suggestive of congenital toxoplasmosis (abortion, a child with congenital anomaly) and history of exposure for the possible associated factors were recorded from all women enrolled in the study using a structured questionnaire. A full verbal explanation about the study was given by the investigators to all voluntary participants.

Laboratory investigations

ELISA test: The IgM and IgG antibodies against *Toxoplasma gondii* in the 388 collected serum samples were determined. The IgM antibodies were quantitatively determined by commercially available kits (SERION ELISA classic *Toxoplasma gondii* IgM, Order Nr.:ESR110M) according to the manufacturer's instructions. While IgG was quantitatively determined by the conventional ELISA at a concentration of 20 μL mL^{-1} coating buffer, pH 9.6, the whole soluble tachyzoites antigen was prepared[8] and the test procedure was done according to the methods described by Shaapan *et al.*[9]. The absorbance of the controls and samples was determined at 450 nm. Positive and negative control sera were included in each run and the cut off value was calculated as the mean OD of the positive controls+3 standard deviations while samples were considered positive if OD value is above cut off value[10].

Avidity ELISA: The avidity of *T. gondii*-specific IgG antibodies was determined as previously described by Hedman *et al.*[11], with some modifications, the 96 well microtiter plates (Nunc, Denmark) were coated with whole soluble tachyzoites

antigen (5 µg mL^{-1}) in carbonate-bicarbonate buffer (pH 9.6) overnight at 4°C. Plates were washed for three times with PBST (PBS, 0.05% tween20) and then sera diluted in PBST (1:200) were added in duplicate rows (row A and row B). After incubation for 1 h at 37°C, row B was washed three times with PBST and row A was washed three times with PBST containing 6 M urea and a fourth time with PBST. Then anti-human IgG conjugated with horseradish peroxidase (Dako, Denmark) at the dilution of 1/1000 in PBST was added and incubated for 1 h, followed by addition of the ortho-phenylene-diamine (OPD), (Merck, Germany) substrate. The reaction was stopped and the absorbance (Abs) was read by an automated ELISA reader (BIOTEC, LX800, USA) at 492 nm. Avidity index (AI; %) was calculated as the result of Abs of wells washed with PBST containing urea (U+), divided by the Abs of wells washed with PBST (U−) and multiplied with 100, based on the equation:

$$AI = \frac{Abs(U+)}{Abs(U-)} \times 100$$

Data analysis and interpretation: Using Statistical Package for the Social Sciences (SPSS version 20.0) the groups were characterized according to the target variables using descriptive statistical analysis methods. Prevalence of toxoplasmosis was defined as the percentage of positive cases for serological tests. Associations between toxoplasmosis and possible risk factors were tested with Chi-square test.

Ethical considerations: The study was ethically cleared by ethical review board of National Research Centre (No. 0226 at May, 2017). The study subjects were informed about the study and written informed consents were obtained from all of the participants before collecting blood samples. Participation in the study was on voluntary basis and study subjects were free to withdraw from the study before and after collection of blood samples without losing any of the benefits they were supposed to obtain from the hospital.

RESULTS

Socio-demographic characteristics: The average age of the woman who took part in this investigation was 27±3.4. Majority of the study participants, 270(69.6%) were rural residents. Regarding their educational status, 119 (30.7%) of the respondents had a secondary school certificate. Occupation wise, the majority of the 262 (67.5%) was housewives (Table 1).

History of exposures to different *T. gondii* infection risk factors: Comparable results of sero-positivity were obtained among 2nd and 3rd trimesters with prevalence of 33.1 and 35.8%, respectively, 24.0% positive cases were from multigravida category, 42.5% of women with history of abortion were positive for *Toxoplasma*, 35.4% of pregnant women who had cats in their households and 27.4% in close contact with livestock were found to be positive for *T. gondii*. About 71.6% of those who took immunosuppressive drugs, 34.5% of women using river or well water as a source of drinking water, 24.4% had a habit of eating raw or undercooked vegetables and a blood transfusion history cases were 30.8% positive for infection by *T. gondii* (Table 2).

Total prevalence of *T. gondii*: The total sero-prevalence of *T. gondii* through the investigated pregnant women was 20.4% (79/388). The acute infection group I revealed presence of both toxoplasma IgM and IgG antibodies and had a low IgG avidity index (AI≤50) included 28 women (7.2%) while the chronic infection group III showed negative IgM and positive IgG with high IgG avidity index (AI≥50) included 36 women (9.3%) (Table 3).

Table 1: Seroprevalence of *T. gondii* infection in relation to socio-demographic characteristics among examined pregnant women (N = 388)

Variables	No examined (%)	Positive (%)	Negative (%)	p-value
Age (year)				
15-19	9 (2.3)	0 (00.0)	9 (100.0)	0.035*
20-24	108 (27.8)	17 (15.7)	91 (84.3)	
25-29	176 (45.4)	27 (15.3)	149 (84.7)	
30-34	62 (16.0)	19 (30.6)	43 (69.4)	
35-39	26 (6.7)	11 (42.3)	15 (57.7)	
40-44	7 (1.8)	5 (71.4)	2 (28.6)	
Residence				
Rural	270 (69.6)	70 (25.9)	200 (74.1)	0.011*
Urban	118 (30.4)	9 (7.6)	109 (92.4)	
Education				
Illiterate	106 (27.3)	29 (27.4)	77 (72.6)	0.243
Read and write	93 (24.0)	20 (21.5)	73 (78.5)	
High school	119 (30.7)	21 (17.6)	98 (82.4)	
Collage	70 (18.0)	9 (12.9)	17 (85.0)	
Occupation				
House wife	262 (67.5)	53 (20.3)	209 (79.7)	0.352
Different works (Teacher, nurse etc.)	126 (32.5)	26 (20.6)	100 (79.4)	

Table 2: Seroprevalence of *T. gondii* infection in relation to exposure to different risk factors of *T. gondii* infection among examined pregnant women (N = 388)

Variables	No examined (%)	Positive (%)	Negative (%)	p-value
Gestational age				
1st trimester				
History of normal delivery	111 (28.6)	0 (0.0)	111(100.0)	0.047*
History of abortion	47 (12.1)			
2nd trimester	121 (31.2)	40 (33.1)	81 (66.9)	
3rd trimester	109 (28.1)	39 (35.8)	70 (64.2)	
Gravidity				
Prima gravidae	118 (30.4)	20 (16.9)	98 (83.1)	0.007*
2nd gravidae	99 (25.5)	18 (18.2)	81 (81.8)	
Multigravida	171 (44.1)	41 (24.0)	130 (76.0)	
History of abortion				
Yes	47 (12.1)	20 (42.5)	27 (57.5)	0.001*
No	341 (87.9)	59 (17.3)	282 (82.7)	
History of intake of immunosuppressive drugs				
Yes	67 (17.3)	48 (71.6)	19 (28.4)	0.001*
No	321 (82.7)	31 (9.6)	290 (90.4)	
Consumption of raw/undercooked vegetable				
Yes	78 (20 .1)	19 (24.4)	59 (75.6)	0.682
No	310 (79.9)	60 (19.4)	250 (80.6)	
Presence of cat at home				
Yes	130 (33.5)	46 (35.4)	84 (64.6)	0.000*
No	258 (66.5)	33 (12.8)	225 (87.2)	
Animal breeding (e.g., sheep, goat, cow, donkey)				
Yes	78(45.9)	49 (27.4)	129 (72.6)	0.04*
No	210 (54.1)	29 (13.8)	181 (86.2)	
Source of drinking water				
Pipe	333 (85.8)	64 (19.2)	269 (80.8)	0.349
Well/river	55 (14.2)	19 (34.5)	36 (65.5)	
Blood transfusion				
Yes	13 (3.4)	4 (30.8)	9 (69.2)	0.257
No	375 (96.6)	75 (20.0)	300 (80.0)	

Table 3: Detection of specific *T. gondii* serological markers in sera of the investigated pregnant and aborted women

Groups	No.	%	Samples	Descriptions	Diagnosis
I	28	7.2	11 P (39.3%) and 17 A (60.7%)	Positive IgM and IgG antibodies and low AV	Acute infection
II	15	3.9	12 P (80%) and 3A (20%)	Positive IgM and IgG antibodies and high AV	Recent non acute infection
III	36	9.3	All P (100%)	Negative IgM and positive IgG antibodies and high AV	Chronic infection
Total	79	20.4		Total	

P: Pregnant women in the 2nd and 3rd trimester, A: Pregnant women in the 1st trimester with history of abortion, AV: IgG Avidity

DISCUSSION

The chance of acquiring acute infection with *T. gondii* is high during pregnancy and the infection would have potential tragic outcomes for the mother, the fetus and newborn despite the fact that it can be prevented[12]. This investigated demonstrated that overall prevalence of toxoplasmosis in examined pregnant women was 20.4%. Nearly the same prevalence (21.3%) was reported in Almadinah Almunawwarah, KSA[13] and among Portuguese population (22%)[14]. Higher prevalence was reported by several researchers; 83.6% in Jimma town, Southwestern Ethiopia[15], 43.6% in Khartoum State, Sudan[16] and 38.8% in the south western region of Saudi Arabia[17]. On the other hand, lower seroprevalence was recorded by several investigators; 18.1% in South Africa[18] and 18.5% at FelegeHiwot Referral Hospital, Bahir Dar town, northwest Ethiopia[19].

In the concurrent work results showed that 35.4% of positive *Toxoplasma* sera had anti-toxoplasma IgM and IgG and low IgG avidity index (AI$\geq$50) (Acute infection group I) and 45.6% had negative anti-toxoplasma IgM and positive IgG and high IgG avidity (AI$\geq$50) (Chronic infection group III). Some parallel studies; reported that 80% sera with acute toxoplasmosis showed low avidity levels and 96% sera in chronic phase of infection showed high avidity index in Qom Province, Iran[20], in Saudi Arabia it was reported that 6 (18.8%) of 32 pregnant women with *T. gondii* IgM seropositive samples had high IgG avidity and 26 (81.3%) had low IgG avidity indicating acute toxoplasmosis[21].

The findings of the present study showed significant association between *T. gondii* positive cases and age of the study participants above 30 years. Results that have been reported from Jimma town, Ethiopia and Brazil have shown statistically significant association between age of pregnant women and seroprevalence of *T. gondii* infection[15,22].

Current results showed that 25.9 and 7.6% of positive toxoplasma cases were from rural and urban settings, respectively with significant difference. Residence was found to be significant in rural areas ($p = 0.001$)[16]. In relation to histories of abortion, there was significant association between occurrences of previous abortions (42.5%) and seroprevalence for toxoplasmosis in the investigated positive cases (17.3%). These results agree with those from Sudan and India[16,23].

Concerning risk factors; in our study, 71.6% of the positive *Toxoplasma* cases taking immunosuppressive drugs were positive for *Toxoplasma*. Similar results were obtained from Saudi Arabia[24] and Sri Lankan[25] (non-immune women).

In the present study animal rearing accounted for 27.4% of pregnant women who were in close contact with animals and there is a considerable relation between *T. gondii* infection and presence of domestic cats at home (35.4%). This finding is in agreement with studies reported from Ethiopia and Taiwan[15,26]. This differences and variations among reported studies could be the hazard of contracting *T. gondii* infection might not just be the presence of cats in the households, but it could be a contact of cats' fecal material while gardening. The absence of a statistically significant relationship between the sero-prevalence of *Toxoplasma* infection and some potential factors (blood transfusion, consumption of raw/undercooked vegetable and drinking water source) does not mean that they have no influence on the transmission of toxoplasmosis[27]. However, it may suggested that such factors play a limited role in the study area for the transmission of the parasite in the studied subjects.

CONCLUSION

It can be concluded that the sero-prevalence of *T. gondii* infection among the pregnant women in Giza governorate was relatively high. This finding revealed that exposure to *T. gondii* infection may increase the risk of premature termination of pregnancy. In this study, several risk factors that could lead to an increase in the prospect of *T. gondii* infection in the pregnant women we redetected, including age, rural residence, an increase in gravidity and gestational age, intake of immunosuppressive drugs, history of previous abortion and contact with cat and farm animals. Therefore, implementation of regular serological testing during pregnancy, health education towards avoiding contact with cats fecal material during cleaning and gardening should be emphasized by health extension workers and other public health professionals for prevention of the disease.

SIGNIFICANCE STATEMENT

This study introduce good diagnostic efficacy for detection of toxoplasmosis in pregnant women with differentiation between the recent acute and chronic infected cases using the IgG avidity test is beneficial in differentiating recent from old *Toxoplasma* infection. So, the authors suggest screening of *T. gondii* infection for all pregnant women by detection IgG, IgM antibodies and additionally advice apply IgG avidity test (AV) to avoid risk of premature termination of pregnancy and also to look any risk factors related to the infection.

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