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Research Article
Toxoplasma Gondii Infection and Associated Sociodemographic and Behavioral Risk Factors among Blood Donors

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Abstract

Background and Objective: *Toxoplasma gondii* is widely spread protozoan parasite transmitted to human through various routes including blood transfusion. There is scarce information about the epidemiology of *T. gondii* in blood donors in Egypt. Therefore, this study aimed to determine the prevalence of *T. gondii* and associated sociodemographic and behavioral risk factors in a population of healthy blood donors of Beni-Suef Governorate and detect the possible risk for transmitting toxoplasmosis to blood recipients in a cross-sectional study. **Materials and Methods:** Blood samples were collected within 7 months from a total of 276 blood donors after taking questionnaire sheet to explore different risk factors. All participants were tested for presence of *T. gondii* IgG and IgM immunoglobulins using ELISA. Also seropositives were tested for IgG avidity test for confirming acute infection. **Results:** Out of 276 participants, 150 (54.3%) and 17 (6.1%) tested positive for anti-*Toxoplasma* IgG and IgM, respectively. Low avidity antibodies were detected in 6 (4%) out of 150 seropositive samples by IgG avidity test suggesting recent infection. *Toxoplasma* seropositivity was statistically significant in male patients, those living in rural areas and those eating improperly cooked/processed meat. However, there were no significant differences with age, educational level, blood group type or Rh factor, eating raw vegetables, contact with soil or cats. **Conclusion:** *Toxoplasma* seropositivity in blood donors may be an indication for its general prevalence in Beni-Suef Governorate. Presence of low avidity antibodies drew attention to the possible transmission of toxoplasmosis through blood transfusion to seronegative recipients and highlights the importance of *Toxoplasma* screening as a preceding test before donation of blood.

Key words: *Toxoplasma gondii*, sociodemographic, risk factors, immunoglobulin, seroprevalence, blood donors

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Blood transfusion although being a life-saving therapeutic intervention, it can transmit several infectious agents such as bacteria, viruses, fungi and parasites including *Toxoplasma gondii* [1]. *T. gondii* is a zoonotic protozoan parasite causing abortions and stillbirths in wide variety of intermediate host animals and human [2,3]. Although, most *Toxoplasma* infections occur by ingestion of tissue cysts in undercooked meat or mature oocysts from soil or by congenital transmission, blood or leukocytes transfusion can also transmits the parasite to the susceptible recipients[4-6].

Blood donors carry the risk of transmitting *T. gondii* to the blood recipients only in the acute parasitaemic phase where, the transient circulating tachyzoite stage remains alive in the buffy coat or citrated blood for up to 50 days[7] at 4°C. Worthily noted, some patients are exposed to regular multi-transfusion of blood in cases of aplastic anemia, sickle cell anemia and thalassemia's and some blood recipients may be infant, pregnant women or immunosuppressed patients. So, they are at continuous risk for *Toxoplasma* infection in whom the disease will be serious or even fatal[8,9].

The majority of *Toxoplasma* infections in the acute phase are asymptomatic with exceptional cervical lymphadenopathy in 10% of the cases[10]. So, diagnosis of toxoplasmosis in healthy blood donors cannot rely exclusively on clinical manifestations but should be confirmed by other parasitological or serological assessments[11]. Several serological tests had been categorized to detect anti-*Toxoplasma* IgG and IgM immunoglobulins with the issue of distinguishing between recent and chronic infection being problematic due to persistence of anti-*Toxoplasma* IgM for many months or years after the end of the acute phase[12].

Meanwhile, the measurement of IgG avidity has proved a useful procedure in diagnosing recent *Toxoplasma* infection[13]. It is based on the existence of low binding affinity of an antibody for its antigen after primary antigenic exposure followed by subsequent increase after weeks or months of antigen-driven B-cell selection. Urea is used as dissociating agent to break up weak antigen-antibody bonds and as a result, low avidity antibodies will be indicative for acute infection while high avidity antibodies is associated with chronic infection[14].

The prevalence of *Toxoplasma* infection in healthy blood donors varies in different countries depending on the rate of infection in the community, the distinct geographic and temporal differences in transmission patterns[6]. Prior studies conducted in Egypt reported considerable variations in the prevalence of *Toxoplasma* in blood donors which ranged[15-17] from 19.5-65%. According to the general standards for blood transfusion in Egypt, all donated blood are routinely screened for some of the most important microbial agents such as Hepatitis B virus, Hepatitis C virus, *Treponema pallidum* and HIV[18], while, serological diagnosis of *T. gondii* is not included among those routine screening tests.

The present study was initially proposed to determine the prevalence of *Toxoplasma* infection among healthy blood donors in Beni-Suef Governorate, Egypt using ELISA IgG, IgM and IgG avidity to show to what extent *Toxoplasma* sero-positive blood donors may transmit the infection to the blood recipients and find out if there is a necessity for screening of *T. gondii* in blood donors. Also, to estimate different risk factors associated with *T. gondii* infection to define the epidemiology of this infection in healthy blood donors.

MATERIALS AND METHODS

Study design and study population: A cross sectional study was conducted on 276 blood donors attending the blood bank of Beni-Suef General Hospital, Egypt in the period from May to December 2016. All blood donors who accepted to participate in the study were healthy volunteers with no symptoms suggestive of *Toxoplasma* infection, with age of 18 years and older and of both sexes. All participants were subjected to standardized questionnaire to explore socio-demographic and behavioral data. Also, the correlation with ABO blood grouping and Rh factor was analyzed.

Samples collection and preparation: Briefly, 5 mL blood was aseptically collected from each blood donor, sera samples were prepared and stored at -20°C in sterile Eppendorf tubes until serologically assayed[19]. As a routine in the blood bank, all donated blood were assayed for complete blood count, blood grouping type and Rh factor. Also, they were screened for antibodies against human immunodeficiency virus, hepatitis C virus, and *Treponema pallidum* in addition to hepatitis B virus surface antigen[20]. Any positive blood sample for any of the previously mentioned infections was condemned and excluded from the study. The storage of different samples in the blood bank was as follows: whole blood at 2-6°C for a maximum 35 days, plasma at -80°C for 1 year, while platelets rich plasma at -5°C for 6 h.

Toxoplasma IgG and IgM serological assay: ELISA96-well microtiter plates were sensitized with 100 µL sonicated *Toxoplasma* antigen/well (provided from Zoonotic Disease Department, National Research Center) at a concentration of

20 µL mL^{-1} coating buffer, pH 9.6. The procedure was done according to Shaapan *et al.*[21]. Absorbance of the samples and controls was determined at 450 nm by ELISA microplate reader. Cut off value was calculated as mean OD of the positive controls+3 standard deviation and the samples were considered positive if OD value is above cut off value[22].

Toxoplasma IgG avidity: All seropositive samples were also screened by Nova Lisa *Toxoplasma gondii* IgG avidity kit (Nova Tec immunodiagnostic GmbH, Germany). Each diluted serum sample, performance control and blank was dispensed twice into 2 separate wells with following the manufacturer instructions. For each sample, the ratio between absorbance of the well dispensed with avidity reagent and the absorbance of the same well dispensed with washing buffer was calculated multiplied by 100. Serum samples with high avidity (>40%) were regarded as chronic infection while low avidity (<40%) as acute infection.

Statistical analysis: Statistical analysis of results and data presentation was done using the SPSS-23 (IBM, Somers, NY, USA) software. Numerical data were presented as Mean±SD while categorical data were expressed as number and percentage. The association between any two qualitative variables was studied by Chi-square. p-value was statistically significant if $\leq$0.05.

Ethical approval: The study was approved by National Research Centre Ethics Committee. Written consents were taken from all the participants in the study before collecting the data, and all had the right to withdraw from the study if they refused to participate.

RESULTS

This study was carried out on 276 healthy blood donors with age range between 18 and 51 years and mean of 29.7 (±8.2) years. Males represented 78.3% of the participants with mean age of 29±7.8 years, while females were 21.7% with mean age 31±9.4 years. About, 121 (43.8%) were from urban areas while 155 (56.2%) were from rural areas. Other socio-demographic and behavioral data of the studied populations were described.

Serological screening of *Toxoplasma* IgG antibodies showed that 150 (54.3%) out of 276 blood donors were *Toxoplasma* seropositive. Regarding sociodemographic factors, males showed higher seropositivity for *Toxoplasma* IgG than females (66 and 34%, respectively) with statistical significant difference (p-value $\leq$0.005). Also, living in rural areas showed IgG seropositivity more than urban areas (61.3 and 38.7%, respectively) with statistical significant difference (p-value = 0.03). Subjects aged 18-24 years showed the highest IgG seropositivity compared with the other two age groups with no statistical significance. Educational level didn't also show any statistical significance (p-value = 0.2). Blood group A was the most frequent group (48.7%), while AB was the least frequent blood group (8%) with no detected correlation between *Toxoplasma* seropositivity and ABO blood group or Rh factor (p-value >0.05). Regarding behavioral risk factors, eating undercooked/processed meat was the only factor that showed statistical significance with *Toxoplasma* IgG seropositivity (p-value = 0.01). Other factors such as contact with cats or soil and eating raw vegetables revealed no statistical significant association (p-value >0.05) (Table 1).

The total *Toxoplasma* IgM positive samples were 17 (6.1%), from which 2 cases (0.7%) were seropositive for IgM only while the remaining 15 (5.4%) cases were seropositive for both IgM and IgG. By IgG avidity testing, 6 (4%) cases out of 150 seropositive samples for anti-*T. gondii* IgG antibodies showed low avidity antibodies suggestive of recent infection while the remaining 144 (96%) seropositive cases showed high avidity antibodies indicative of chronic infection. From the total 17 cases positive for IgM, 6 cases showed low avidity antibodies and the remaining 11 cases were considered as a false positive IgM (Table 2).

DISCUSSION

Blood transfusion is one of the potential routes for *Toxoplasma* transmission representing a major threat especially for immunosuppressed patients, multiple blood transfusion recipients, fetus and pregnant women [23]. Presence of tachyzoites in blood during the course of active infection plus their ability to survive in the stored blood for several weeks is a real danger for blood recipients. So, it is crucial to screen for *Toxoplasma* infection in blood donors and make efforts to reduce its transmission through blood transfusion[24]. The current study showed an overall *Toxoplasma* seroprevalence of 54.3% in healthy blood donors. In comparison to previous studies conducted on blood donors in Egypt, this rate of seroprevalence was slightly lower than the prevalence in Mansoura and Alexandria Governorates, 59.6 and 65.3%[16,17], respectively; while higher[15,25] than 19.5 and 33.6%, respectively.

Table 1: Seroprevalence of *T. gondii* IgG antibodies in 276 blood samples of donors in correlation with some sociodemographic and behavioral risk factors

			IgG ELISA				
	Total (n = 276)		Seropositive		Seronegative		
Characteristics	No.	Percentage	No.	Percentage	No.	Percentage	p-value
Gender							
Male	216	78.3	99	66.0	117	92.9	≤0.005*
Female	60	21.7	51	34.0	9	7.1	
Age group (year)							
18-24	96	34.8	57	38.0	39	31.0	0.4
25-34	96	34.8	48	32.0	48	38 .0	
35-51	84	30.4	45	30.0	39	31.0	
Residence							
Urban	121	43.8	58	38.7	63	50.0	0.03*
Rural	155	56.2	92	61.3	63	50.0	
Educational level							
Non-educated	13	4.7	10	6.7	3	2.4	0.2
Mid-educated	125	45.3	65	43.3	60	47.6	
High-educated	138	50.0	75	50.0	63	50.0	
Blood group type							
A	127	46.0	73	48.7	54	42.8	0.3
B	89	32.3	50	33.3	39	31.0	
AB	24	8.7	12	8.0	12	9.5	
O	36	13.0	15	10.0	21	16.7	
Rh factor							
Positive	250	90.6	138	92.0	112	88.9	0.2
Negative	26	9.4	12	8.0	14	11.1	
Eating undercooked meat							
Yes	124	44.9	77	51.3	47	37.3	0.01*
No	152	55.1	73	48.7	79	62.7	
Eating raw vegetables							
Yes	54	19.6	30	20.0	24	19.0	0.4
No	222	80.4	120	80.0	102	81.0	
Contact with cats							
Yes	40	14.5	23	15.3	17	13.5	0.3
No	236	85.5	127	84.7	109	86.5	
Contact with soil							
Yes	111	40.2	60	40.0	51	40.5	0.5
No	165	59.8	90	60.0	75	59.5	

*Significant p-value

Table 2: Results of IgG avidity used in the study in comparison to IgM and IgG ELISA

		Avidity test	
Serological test	No. (%)	Low avidity	High avidity
+ve *Toxoplasma* anti-IgM -ve *Toxoplasma* anti-IgG	2 (0.7)	0%	0%
+ve *Toxoplasma* anti-IgM +ve *Toxoplasma* anti-IgG	15 (5.4)	4/150 (2.7%)	11/150 (7.3%)
-ve *Toxoplasma* anti IgM +ve *Toxoplasma* anti-IgG	135 (48.9)	2/150 (1.3%)	133/150 (88.7%)
Total	276 (100)	6/150 (4%)	144/150 (96%)

Several studies conducted worldwide on blood donors showed lower seroprevalence than the current study; Saudi Arabia (52.1%)[26], Czech Republic (33.1%)[27], Malaysia (28.1%)[28], Mexico (7.4%)[6], India (19.6%)[29], Taiwan (9.3%),[30] and Iran (28.8 and 34.4%)[1,31]. This wide variability in *T. gondii* infection prevalence rates may be attributed to differences in cultural and social habits, geographic and environmental factors[6].

It is quite obvious that the presence of IgM antibodies alone (IgM positive/IgG negative) is rarely seen due to the short period between appearance of IgM and IgG. This finding was consistent with the current results where IgM were detected alone in 0.7% while IgM and IgG were found in 5.4% of samples.

Total IgM percent (6.1%) in the present study was, however, parallel to the rate (6%) recorded by El-Sayed *et al.*[25] in Egypt and higher than other studies conducted on blood

donors worldwide; 4.1% in Saudi Arabia[26], 2.4% in Czech Republic[27], 0.36% in India[29], 1.9% in Mexico[6] and 3.2% and 1.7 in Iran[1,31].

IgM antibodies detection can't be solely considered as a dependent test for diagnosing acute *Toxoplasma* infection. So, IgG avidity determination test was used as confirmatory test to differentiate between recent and past *Toxoplasma* infection in patient's sera[32]. Low avidity antibodies were detected in the present study in 6 (2.2%) cases denoting recent infection while the remaining 144 (52.2%) showed high avidity antibodies suggestive of chronic infection. The apparent discrepancy in detecting recent infection status by IgM serology (6.1%) versus avidity testing (2.2%) may be explained by many factors; persistence of IgM antibodies for months or even years after the acute infection in some individuals, non-specific binding, presence of rheumatoid factor and antinuclear antibodies causing false positive results[33,34]. However, this small sector of healthy blood donors (2.2%) who showed low avidity antibodies is very dangerous for immuno-suppressed blood recipients and those undertaking immune-suppressive drugs in particular.

Higher seropositivity of *T. gondii* (38%) in the younger age group (18-24 years) in the present study was in accordance with Zainodini *et al.*[31] and Shaddel *et al.*[35] and contrary to other studies that showed higher *Toxoplasma* infection in old age[1,17,27]. The result in this research can be explained by increased consumption of this category of age group for quick insufficiently cooked meat, what's called fast food, which depends on ineffective heating, salting or freezing during meat storage[36]. However, there was no statistical association between seropositivity and age.

Males were higher (66%) than females (34%) regarding *Toxoplasma* seropositivity with statistical significance (p-value $\leq$0.005), which is in accordance with others[30]. However, other studies pointed to higher incidence of *Toxoplasma* in females[1,37,38]. This finding may be due to sampling bias where most participants were males, a common finding in blood donation. Further larger scales ex-matched studies must be performed to explore this relation taking into consideration the typical paucity of females among blood donor populations.

Regarding residence, IgG seropositive results was higher in subjects who live in rural areas with notable significant difference (p-value = 0.03). This finding was in agreement with Elsheikha *et al.*[16] and Mahmoudvand *et al.*[1] and may be due to low socioeconomic standards, lack of hand hygiene before meals, eating unwashed vegetables, drinking unfiltered water and frequent exposure to animal excreta.

There was no significant association between *T. gondii* seropositivity and educational level which was opposite to other studies[38,39]. Although blood group A had the higher (48.7%) *Toxoplasma* seropositive rate in the present study, it was of no significant difference. However, Shaddel *et al.*[35] and Mahmoudvand *et al.*[1] reported the significant correlation between blood group B and AB with *T. gondii* seropositivity. In Egypt, another study observed an association between *T. gondii* and blood group O for the first time[16]. However, this association was ascribed by the authors as incidental or biased sampling and needs to be elucidated by further studies.

Undercooked meat consumption was the only behavioral risk factor which is significantly associated with IgG seropositivity of *T. gondii* in this study which was consistent with previous studies[1,37]. Conflicting findings concerning the transmission of *T. gondii* via contact with cats have been reported. Contact with cats was found as a potential risk factor for acquiring toxoplasmosis[6,30], while previous report and the present study did not detect a link between contact with cats and *T. gondii* seropositivity[28]. This may be due to immature excretion of *Toxoplasma* oocysts confirmed by that *Toxoplasma* oocysts were not isolated from the fur of oocyst-excreting cats[40].

CONCLUSION

The present study is the first that detected the prevalence of toxoplasmosis among healthy blood donors in Beni-Suef Governorate, Egypt. The presence of *Toxoplasma* IgM antibodies in patient serum is not always an indication of a recent infection and must be confirmed by other tests especially those that depend on antigen detection. Considering the percentage of low avidity antibodies (2.2%) in the current study, taking into consideration that these individuals might have tachyzoites in their blood, toxoplasmosis should be considered as a significant risk in blood transfusion and should be screened before blood donation.

SIGNIFICANCE STATEMENT

This study revealed that IgG avidity is beneficial in differentiating recent from old *Toxoplasma* infection. The authors suggest screening of *T. gondii* for all blood donors to avoid *Toxoplasma* transmitted transfusion.

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antigen through successive mice-mice tachyzoite passage to be used in ELISA technique.

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