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EVALUATION OF DONATED BLOOD FOR TOXOPLASMOSIS USING ELISA AND PCR IN FAYOUM GOVERNORATE

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Abstract

Toxoplasma gondii is an intracellular zoonotic parasite that affects more than a third of the world population. Among many modes of transmission, blood transfusion is a potential risk for transmitting toxoplasmosis especially to high risk individuals as immunosuppressed persons and pregnant women. This cross-sectional study evaluated the donated blood for *T. gondii* infection using ELISA also the puffy coat was separated and PCR for toxoplasma is done. 100 blood samples were collected from blood banks in Fayoum Governorate.

Key words: Fayoum**,** Donated blood, ELISA, PCR, *Toxoplasma gondii*.

Introduction

Toxoplasma gondii is a zoonotic parasite that can be transmitted by ingestion of oocyst or tissue cyst organ transplantation or blood transfusion or from infected mother to her fetus (Saleh *et al*, 2014). Its manifestations depend on host immune status ranging from asymptomatic or low grade fever, lymphadenopathy, flu-like symptoms, malaise and myalgia in immunocompetent persons to life threatening infection in immunocompromised host, also transplacental infection can lead to abortion, stillbirth or severe congenital anomalies (El Mansouri *et al,* 2007). Generally, people are infected by three principal transmission routes: foodborne transmission as consuming undercooked, contaminated meat (Riffat *et al,* 1978; Haridy *et al,* 2009), zoonotic transmission by ingesting oocysts shed in the feces of infected cats (Al-Kappany *et al,* 2010), and vertical transmission from mother to fetus through the placenta during pregnancy. Besides, *T. gondii* can be transmitted via blood transfusion (Sarwat *et al,* 1993) or organ transplantation (Derouin and Pelloux, 2008).

Toxoplasmosis affects one third of the world human population. The prevalence of infection among Egyptian population varied between 30-60%. Studies that investigate the potential risk of donated blood are relatively sparse. The available studies declared that the prevalence of toxoplasmosis in Egypt is increasing since it was 1% (Rifaat *et al,* 1963) to 19.5 % (Azab *et al,* 1986) and then 65.3% (El-Geddawi *et al,* 2016). Prevalence studies were done in special patient groups as it was 65.5% in hepatic patients (Ghanem *et al,* 2001), 69% in acute or chronic renal failure patients more exposure to dialysis (Aufy *et al,* 2009) and 46% in diabetic patients (Mahmoud *et al,* 2018), toxoplasmosis was higher in males patients (26%) than females (10%) with cancer patients (Abdel Malek *et al,* 2018), and a significantly higher among thalassemia children in need of repeated blood transfusions when compared with healthy ones (El-Tantawy *et al,* 2019).
More than half of seropositive individuals were chronically infected as proved by the presence of bradyzoites in their tissues, parasitaemia can persist up to 1 year after infection, and the organism can survive in citrated whole blood stored at 4°C (blood bank refrigeration temp.) up to 50 days (Robert and Dardé, 2012). The transfused blood went to people with aplastic anemia, thalassemia, pregnant females with anemia, and those immunocompromised with chronic diseases as well as others as having caesarean section (Ismail *et al*, 2014).

The commonest laboratory tests for *Toxoplasma* screening included ELISA and Dot-ELISA for detecting IgM and IgG, indirect haemagglutination (IHA), indirect Immunofluorescent antibody test, lateral flow immunochromatographic assay and PCR (Teimouri *et al,* 2018). Other tests include histological examination of tissues and blood smears, culture and Sabin-Feldman dye test

(Sensini, 2006). Lilian *et al*. (2016) suggested that the people who were at the increased risk of toxoplasmosis, such as immunosuppressed individuals, pregnant women and others in need of blood transfusion should receive *T. gondii* antibody-negative blood components before transfusion and also the leuko-depletion may reduce the risk of transfusion-transmitted toxoplasmosis

This study aimed to evaluate the donated blood for *Toxoplasma gondii* infection using ELISA to detect the seropositive ones, also the puffy coat was separated and PCR evaluated the potential infectivity of the blood.

Materials and Methods

The current study was carried out over the year 2018. The study was approved by the Ethical Committee of Faculty of Medicine, Fayoum University. A total of 200 samples from donated blood were collected, 100 samples from Fayoum General Hospital Blood Bank, & 100 samples from Fayoum University Hospital Blood Bank. Sample size was calculated by using the Schwartz formula (Adoubryn *et al*, 2004) with a reference prevalence of 60%, & 95% confidence level. Inclusion criteria were those aged between 18-60, healthy, and average weight over 50Kg.

All samples were labeled with name, age, sex, occupation, residence, Rh type and blood group. Serum and buffy coat were separated and stored at -20°C till further analysis by:-

Serological testing: All serum samples were collected and stored at -20^{o}C until analysis. The commercial Sandwich ELISA Kit (OriGene Te-chnologies, Inc., USA) was used based on the manufacturer's instructions. Index value was ob-tained for both IgG and IgM. An index value $\leq$ 0.9IU/mL was regarded as negative result, while the equivocal range was defined between 0.9 and 1.1 IU/mL and index value greater than 1.1 IU/mL was considered as positive result for both IgG and IgM.

Molecular diagnosis: ELISA positive samples were undergone buffy coat separation for real time PCR. Detection of B1 gene in *Toxoplasma* Genome proved to be the highest sensitivity (Tarak *et al*, 2010). Puffy coat was prepared using phosphate buffer saline **(**Boyum, 1976). The DNA was isolated from the buffy coat of antibody positive sample, using the phenol-chloroform extraction method. For DNA extraction the (NORGEN BIOTEK Corp., Canada) was used. 100ul from the puffy coat was used. DNA was eluted with 100µl of elution solution. The oligonucleotide sequences for analysis of the B1 gene were: the forward primer GCATTGCCCGTCCAAACT, Reverse primer AGA CTGTACGGAATGGAGGAA, and the Taq Man probe 6-carboxy-fluorescein–CAACA ACTGCTCTAGCG-Black Hole Quencher 1 (Operon Biotechnologies, Germany).

The Real-time PCR was performed with an ABI PRISM 7000 genetic analyzer. The reaction mixtures (25µl) consisted of 1 X TaqMan PCR master mix (Applied Bio-Systems), 100nM probe, and 900nM (each) primers forwarded and reversed, together with the different samples. Each well also contained 1 internal positive control (IPC) reagent and 1 IPC synthetic DNA (Applied Bio-Systems). Sterile water was used as a negative control, and *T. gondii* purified genomic DNA was used as a positive control.

Amplification conditions comprised 50°C for 2min, initial activation at 95°C for 10 min, and 45 cycles of denaturation at 95°C for 15s and annealing/extension at 60°C for 1min.

Statistical analysis; Data were analyzed using the statistical package (SPSS) version 18. Data were summarized using mean, standard deviation and range for quantitative variables and for qualitative values%. Comparison between groups were done using independent sample T-test and ANOVA test for quantitative test and Chi-square and Fisher test for qualitative variables. P value less than 0.05 were considered significant.

Results

The results were shown in tables (1, 2 & 3) and figure (1).

Table 1: Demographic and other variables of the studied samples

Age in years	< 30	102 (51%)
	≥ 30	98 (49%)
Sex	Male	158 (79 %)
	Female	42 (21%)
Residence	Rural	130 (65 %)
	Urban	70 (35%)
Blood group	A	57 (28.5 %)
	B	59 (29.5 %)
	AB	23 (11.5 %)
	O	61 (30.5%)
Rh factor	Positive	178 (89%)
	Negative	22 (11%)

Table 2: ELISA and PCR results, and inter-correlation.

STUDY GROUPS	ELISA test No. (%)	PCR test result		Chi square	
		Positive No. (%)	Negative No. (%)	X2	P-value
Positive for IgG only	90 (45 %)	3 (3.3)	87 (96.7)	3.2557	0.071
Positive for both IgG and IgM	40 (20 %)	17 (42.5)	23 (57.5)	4.456	0.091
Positive for IgM only	6 (3%)	4 (66.7)	2 (33.3)	42.524	< 0.001*
Total	136 (68 %)	24 (17.6)	112(82.4)		

* Significant

Table 3: Association between different variables.

Variables		Total	IgG positive N=130		P value	IgM +ve N=46		P value	PCR +ve N=24		P value
			No.	%		No.	%		No.	%	
Age	< 30	102	60	58.8	0.062	26	25.5	0.394	16	15.7	0.107
	≥ 30	98	70	71.4		20	20.4		8	8.2	
Sex	Male	158	102	64.5	0.798	30	19	0.010*	20	12.7	0.579
	Female	42	28	66,7		16	38.1		4	9.5	
Resi-dence	Rural	130	112	86.2	0.001*	40	30.8	0.001*	22	16.9	0.01*
	Urban	70	18	25.7		6	8.6		2	2.9	
Blood Group	A	57	33	57.9	0.082	13	22.8	0.984	7	12.3	0.962
	B	59	36	61		13	22.0		6	10.2	
	AB	23	20	87		6	26.0		3	13.0	
	O	61	41	67.2		14	22.9		8	13.1	
Rh type	Positive	178	120	67.4	0.076*	41	23.0	0.970	23	12.9	0.603
	Negative	22	10	45.5		5	22.7		1	4.5	

* Significant

Discussion

Toxoplasma is an obligate intracellular parasite that can infect human by different modes mostly by ingestion. However, blood transfusion during the acute stage of parasitaemia is a potential risk (Klun *et al*, 2011).

In the present study, there is a uniform distribution for age among the study groups. Regarding the sex 79% of the sample population were males, 21% were females with a ratio of 3.8:1, this is due to the social and cultural aspects of the selected population favoring males for blood donation also due to many exclusion criteria for females including pregnancy, lactation, menstruation and increased prevalence of anemia, 65% of the participants were of rural residence and 89% were Rh positive.

Also, the study showed that 45% were positive for IgG only, 3% were IgM positive and 20% were positive for both IgG & IgM with the total of 68% samples were positive for IgG, IgM or both. This high prevalence of *Toxoplasma* seropositivity is alarming when compared to other endemic areas. The varying prevalence of seropositivity in dif-

ferent places of the world, it is noted that Mexico has the lowest prevalence (Alvarado *et al,* 2007) while places like Brazil (Coêlho *et al,* 2003) and Cuba have the highest prevalence (Martin and Garcya, 2003).

In Egypt, the prevalence of toxoplasmosis among general population varied between 30-60% (Nassef *et al,* 2015), however the studies that explore this issue in blood donors are rare, the first study was done in Cairo 1986 and the prevalence was 19.6% (Azab *et al,* 1989), in Mansoura Governorate was 59.6 % (Elsheikha *et al,* 2009), and in Alexandria was 65.3% (El-Geddawi *et al,* 2016). The variations in prevalence among different countries and even in the same country reflected the population habits, socioeconomic status and exposure to different risk factors. The following chart showed the prevalence of *Toxoplasma* seropositivity among donated blood that is the first in Fayoum Governorate- compared to other studies. Ibrahim et al. (2018) reported that the prevalence in Upper Egypt including Fayoum might be zoonotic from naturally infected pigeons and ducks.

Although the great usefulness of serological testing in practical diagnosis of toxoplasmosis, it has many limitations. For example, it may fail to detect specific anti-*Toxoplasma* immunoglobulin (IgG) or IgM during the active phase of *T. gondii* infection, because these antibodies may not be produced until after several weeks of parasitaemia, also the test may fail to detect *T. gondii* infection in certain immunocompromised patients due to failure of immunoglobulins to rise in the patients (Jose, 2002). Use of specific IgM for diagnosis of acute toxoplasmosis is unreliable as they remain in the patient serum for 1 week to 18 months after invasion by the parasite tachyzoites positive for variable time in chronic infection. Also, sensitivity and specificity of IgM were not 100% and false positive and negative results occur. Specific IgG appeared 1 - 2 months after initial infection.

Rising IgG titer and IgG avidity test when IgG titer and IgG avidity test are the reliable methods used for diagnosis of primary infection or reactivation of chronic latent one. Moreover, the presence of immmunoglobulins didn't necessarily mean the infectivity of the blood but the presence of circulating parasites or tachyzoites inside WBCs renders the donated blood potentially infective (Sensini, 2006).

The real-time quantitative PCR technique has high sensitivity and high specificity to detect the presence of the *T. gondii* genome in blood samples. The clearance time for *Toxoplasma* DNA from the blood of patients with acute toxoplasmic lymphadenopathy was estimated to be about 5.5-13 weeks. Based on this, the presence of *Toxoplasma* DNA in the maternal blood most probably indicates a recent infection or an indicator of apparent parasitaemia, which is likely to be clinically significant (De Mendonca, 2018). However, a negative PCR result does not exclude recent infections due to the short period and the low level of parasitaemia.

In the present study, PCR was done to all positive samples with ELISA revealing that in those samples positive for IgG, only 3.3% were positive for PCR, those positive for both IgG and IgM 42.5% were PCR positive and in those positive for IgM only 66.7 % were positive by PCR with a significant P value, this indicated that PCR positivity is more linked to IgM seropositivity despite the previous studies that deny the reliability of association of IgM with acute infection, we found significant association between IgM seropositivity and parasitaemia detected by PCR. These results agreed with Sadooghian *et al,* (2017) and Darwish *et al,* (2019) who found a statistically significant association between IgM positivity and PCR results.

In the present study, the prevalence of IgG seropositivity increased in those aged ≥ 30, while IgM seropositivity and PCR positivity increased in young age <30 year, these results indicated that acute infections was more in young people while chronic infec

tions more in the elderly matching studies (Jumaian, 2005; Kolbekova *et al,* 2007).

In the present study, there is no statistical significant association between IgG-seropositivity and gender but regarding IgM there was a positive association with female sex, which disagreed with Maryam *et al,* (2017) who showed that males were more commonly affected, but most studies showed no predilection for sex in *Toxoplasma* seropositivity (Oluwatoyin *et al,* 2002; Vincent *et al,* 2011). The study showed significant correlation between toxoplasmosis and rural residence (86.2 %), where contact with cat excreta, lack of health education and low socioeconomic standards are risk factors for infection. This agreed with Kawashima *et al.* (2000) and Salibay *et al.* (2008) who found significantly higher seropositivity to toxoplasmosis in rural than urban settings.

The association between toxoplasmosis seropositivity and certain blood groups has been suggested by previous studies with the highest prevalence associated with Blood group AB and lowest with blood group O (Al-Kaysi and Ali, 2010). These studies proposed that B antigen act as receptor for toxoplasma parasite and the expression of glycoconjugates of the ABO system in the gut epithelial cells in 80 % of the population (Rodrigues *et al,* 2011). Other studies showed no significant association with ABO factor (Mattos *et al,* 2008). In the present study, blood group AB gave the highest Toxoplasma-seroprevalence while group A gave the lowest prevalence. This disagreed with others due to several factors. It is possible that the B antigen exerts a small influence on the adherence of *T. gondii* to the gastrointestinal mucosa and its contribution is obscured by the high prevalence of infection in our population, also the molecular variability of the strains and the use of special population in some studies may affect these results (Boulanger *et al,* 2010). Another theory proposed that the certain blood groups provide resistance to certain infections that have surface antigens similar to those on RBCs so the absence of serum antibodies in group AB make the patient vulnerable to such infections, presence of these antibodies as in group O conveys some resistance to these antigens (Khawla, 2013).

In the present study, the seropositivity to IgM and IgG was associated with Rh positive samples, but the presence of serum antibodies in Rh negative samples may convey some resistance to infection the same mechanism as ABO system.

The present study showed that 17.6 % of the donated blood is positive for *Toxoplasma* B1 gene which indicates that blood is potentially infective and can cause deleterious effects in pregnant females and immunocompromised hosts.

The detection of immunoglobulins remains important as a reference for the clinical and epidemiological patient management and counseling. Performance of assays that detect specific IgG is critically important for the physicians to make correct decisions, especially for the pregnant patients (Moghaddas *et al,* 2019). The study also demonstrated that presence of IgM proved important indicator of recent and potentially active infection (Elsheikha *et al,* 2008). It became of outmost importance to develop a rapid, easy, and reliable and of low cost method such as the lateral flow immunochromatographic assay or strip test which can be done by serum or whole blood without need for sophisticated means. The immunochromatographic test is a field applicable assay and is considered a modification of ELISA in which the colloidal gold-labeled antibodies are used in dot blot assays to avoid use of the sometimes-problematic enzyme-labeled detecting antibodies.

Conclusion

Generally speaking, *Toxoplasma gondii* is a worldwide infectious zoonotic disease that causes different pathogenicity.

The present study demonstrated that 68% of donated blood samples were positive for toxoplasmosis and 17.6% were potentially

infective as being PCR positive. It underscores the importance of screening donated blood for toxoplasmosis especially for the vulnerable groups of patients.

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Explanation of figure

Fig. 1: Prevalence of Seropositivity to toxoplasmosis in different studies