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Seroprevalence and Analysis of Some Risk Factors Associated with Human Toxoplasmosis among HIV Patients Attending Bashyer University Teaching Hospital, Sudan

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Abstract:

Background: *Toxoplasma gondii is Apicomlexa coccidian parasite that causes toxoplasmosis, with congenital toxoplasmosis being the most serious form of infection in human. Infection by T. gondii is usually asymptomatic in immuno-competent human. In pregnant women, the disease is often asymptomatic or have only mild*

symptoms, infection may cause spontaneous abortion, still birth, or serious foetal damage.

***Objectives**: A serological survey was carried out in Bashayer University Hospital, Khartoum, Sudan to assess T. gondii infection rates and risk factors among HIV-positive and HIV-negative people.*

***Materials and Methods** This cross-sectional hospital based survey was undertaken during September 2014-February 2015 using Latex Agglutination Test (LAT) and ELISA-IgM technique.*

***Results**: Antibodies to T. gondii were detected in 36 (43.9%) of the 82 individuals freely agreed to participate in this study. Although the difference was not significant (P>0.05), the overall T. gondii seroprevalence was higher in males (47.6%) than females (40.0%), older (54.5%) than younger (36.7%), married (44.3%) than single (42.9%) and women with no history of abortion (45.5%) than those with history of abortion (27.3%). T. gondii seropositivity was higher in HIV-negative (46.9%) than HIV-positive (42.0%). People with lower haemoglobin concentration (47.6%) and those with lower total white blood cells counts (46.8%). HIV-positive revealed significantly (p=0.044) higher LAT-seropositivity, while HIV-negative revealed significantly (p=0.007) higher ELISA-IgM seropositivity. The univariate analysis showed that, the risk factors that significantly associated with both T. gondii LAT and ELISA IgM seropositive were AIDS and Hb concentration (p=0.044, 0.007 and 0.038) respectively. The multivariate analysis revealed no significant association between the retested significant risk factors with T. gondii LAT seropositivity (p>0.05). However, increasing odds ratios were recorded for Age ≥40 years (odds=1.693, 95% CI=0.631-4.537), HIV-positive (odds=1.837, 95% CI=0.583-5.786) and low haemoglobin concentration (odds=2.262, 95% CI=0.780-6.559). The multivariate analysis showedhighly significant association between HIV-negative persons and anti-T. gondii IgM antibodies seropositivity (p=0.011, odds=4.280, 95% CI=1.399-13.094). Although no significant association (p>0.05), increasing odds ratio was recorded for single persons with T. gondii IgM antibodies seropositivity (odds=1.429, 95% CI=0.432-4.731).*

***Conclusion:** T. gondii infection is widely and equally prevalent in HIV-negative and HIV-positive people, but specific*

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measures must be taken by the HIV-patients and their health-care providers to decrease the risk of acquiring infection or reactivate a latent one.

Key words: Toxoplasma gondii, HIV, Seroprevalence, Haemoglobin, ELISA.

Introduction

Toxoplasma gondii is one of the most important zoonotic foods born pathogen worldwide (1 - 3). Over one third of human population was seropositive (4). People with a weakened immune system, such as those infected with HIV or pregnant women, may become seriously ill, and infection can occasionally be fatal (5 - 8). Seropositive women prior to pregnancy are protected from transmitting the infection to their foetuses (7). Exceptions to this rule have been reported in women with an immunocompromised state (9) and acute infections occurring shortly before conception (10 - 12). Latent *T. gondii* infection may reactivate in HIV-infected humans with encephalitis and neurologic diseases (13 - 15) and can affect the heart, liver and eyes as well as congenital transmission; these infected children usually have HIV as well (16). Risk of infection was found to be increased with age, low educational levels and in individuals who have soil-related occupations (17). Food-borne toxoplasmosis may result from exposure to different stages of *T. gondii*, in particular from the ingestion of tissue cyst or tachyzoites contained in meat or primary offal (viscera) of different animals (1, 2, 18). Around 45.3% of our food animals (Sheep, goats, camels and cattle) in the Sudan were seropositive for *T. gondii* (19).Although *T. gondii* infection was reported early (20) in the Sudan, scientific reports on Human toxoplasmosis were very few. Though few, most of them were in pregnant women (21 - 28). However, nowadays toxoplasmosis

becomes a serious infection in all sexes and ages with the emerging of several causes of immunosuppression, particularly AIDS. Detailed recent data of groups at risk are missing in the Sudan. Therefore, the present study was planned to assess the seroprevalence of toxoplasmosis and associated risk factors (age, sex, marital status, abortion, Haemoglobin concentration and Total white blood cell counts) among HIV positive people as study group and HIV negative people as control group.

Materials and Methods

The Study Area: Bashayer University Hospital is located in Jabal Aolia locality. This locality as one of the seven localities of the Khartoum State found in the Southern part of the capital State of the Sudan (fig. 1).

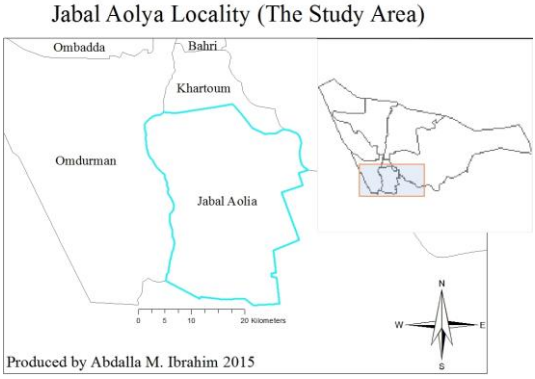

Fig.1: The study area.

The Study Population: Patients enrolled in the HIV/AIDS treatment and follow up programme at the hospital during September 2014 - February 2015 were asked to participate in this study as study group. The control group was consisting of apparently healthy people (workers and visitors) present in the same hospital at the same time. After a verbal consent, a short interview containing history of HIV infection, AIDS treatment,

health status, sex, age and social status (marriage) as well as history of abortion for women.

Ethical Considerations: This study was approved by the ethical committee of the Gharb El-Nile College of Medical laboratory Sciences and Bashayer University Teaching Hospital Khartoum, Sudan. The aim of the study was clearly explained to all individuals participated inthe study. After that, a verbal consent was obtainedfrom all of them before sampling.

Samples Collection: About *1millimeter(ml)* blood with EDTA was collected for Haemoglobin concentration and Total White Blood Cells counts. After that, four to five *mls*of venous blood were collected from each participant in plain vacutainers using sterile disposable syringes under aseptic condition and allowed to clot. Samples were then centrifuged at 1500rpm for 10 minutes to separate serum. Clear sera were carefully collected, aliquot into cryotubes and kept frozen at -20ºC until tested.

Anti-*T. gondii* Antibody Detection: Each serum was tested for presence of *T. gondii* specific IgG and IgM using Latex Agglutination Test (LAT) and Enzyme Linked Immunosorbent Assay (ELISA-IgM) respectively.

Latex Agglutination Test (LAT): Testing was performed using the Latex Agglutination Test kits *(fortress® diagnostics Limited, UK)*. The test was performed according to the manufacturer's instructions. Briefly, the samples were tested in screening dilution 1:16 in physiological saline. Positive samples were retested using serial double dilutions from 1:16 (1:32, 1:64 and 1:128). A positive and negative control serum was tested alongside the samples. Antibody titres of ≥1:16 were considered positive. The antibody titer is the highest dilution with clear evidence of agglutination.

Enzyme Linked Immunosorbent Assay (ELISA): Toxoplasma IgM EIA test Kit *(Foresight® ACON Laboratories Inc. USA)* was used in this study. ELISA results were recorded using an automatic microplatereaderas a measure of optical densities of thereaction intensity of *T. gondii*antigen and serum anti-*T. gondii*antibodies. Cut-off points and antibody indexcalculations were done according to manufacturers' recommendation to categories seropositive (antibody index ≥1.1) and sero-negative (antibody index <1.1). All serum samples with intensity of antibody index ≥2.0 were classified as high sero-positive rate.

Interpretation of results: A negative result indicates that there was no prior exposure to *Toxoplasma gondii*. These individuals are presumed to be susceptible to a primary infection. A positive result indicates that there was a prior exposure at some undetermined time to *Toxoplasma gondii*. A highly antibody titration/index may indicate acute or recent infection.

Statistical Analysis: Data obtained were entered into computer database. Statistical Package for Social Science (SPSS) software version 17 was used. *T. gondii* antibody prevalence was compared across the investigated variables using chi-square test and logistic regression. A value of $P \leq 0.05$ was considered significant.

Mapping: Maps were produced using *Arc GIS version 10.2.2 (ESRI, Redlands, California, USA)* to show the study area.

Results

Descriptive Statistic:

A total of 82 adult persons (50 HIV-positive and 32 HIV-negative) were freely agreed to participate in this study. The majority (74.4%) of them (40 male and 42 female) is married. The age of the tested people was ranging from 18 to 63 years old with mean age of (36.3±9.5). Their Haemoglobin concentration was ranging from 7 to 14 g/dl (50-95%) with mean of (10.7±1.3). Total white blood cells count (TWBC) was 2800 to 14500 with mean of (5263±2080). Repeated abortion (2 to 3 times) was reported in the present study (Table 1). The highest IgM index recorded in this study was 17 with mean of (2.74±4.25).

Table 1: Descriptive Statistics of the samples tested for *T. gondii* antibodies.

	Age (Yr)	Hb (g/dl)	Hb (%)	TWBCs	Abortion	IgM Index
N	**82**	**80**	**80**	**80**	**11**	**18**
Mean±SD	36.3±9.5	10.7±1.3	72.7±8.8	5263±2080	1.5±0.69	2.74±4.25
Mode	35	10	70	3700	1	1
Range	18-63	7-14	50-95	2800-14500	1-3	1-17

Seroprevalence of *T. gondii* infection:

The overall seroprevalence of *T. gondii* infection was 36 (43.9%). Twenty-two percent (18 persons) were seropositive for IgM and 26 persons (31.7%) were seropositive for LAT (Table 2).

Table 2: The overall seroprevalence of *T. gondii* infection using LAT and ELISA IgM tests.

Test	N-ve	P+ve
LAT	56 (68.3%)	26 (31.7%)
ELISA IgM	64 (78.0%)	18 (22.0%)
Overall Prevalence Rate	**46 (56.1%)**	**36 (43.9%)**

Eight samples (44.4%) out of the 18 IgM seropositive samples were found to be positive by both LAT and ELISA-IgM (Table 3). Five (62.5%)of them were HIV-positive.There were no statistically significant differences (p>0.05) in the distribution of antibody level(antibody titration or IgM index) among the seropositive samples in the two diagnostic tests (Table 4).

Table 3: The level of agreement between ELISA IgM and LAT in the detection of *T. gondii* infection in people from the Khartoum State.

		Toxo-LAT Result		Total (%)	P value
		N-ve (%)	P+ve (%)		
Toxo-IgM Result	N-ve	46 (71.9)	18 (28.1)	**64 (78.0)**	**0.189**
	P+ve	10 (55.6)	8 (44.4)	**18 (22.0)**	
Total		**56 (68.3)**	**26 (31.7)**	**82 (100)**	

*Kappa value=0.141

Table 4: Distribution of antibody titration among anti-*T. gondii*IgM seropositivity.

		LAT titration			Total	P value
		1:16	1:32	1:64		
Toxo-IgM Result	N-ve	16 (88.9)	1 (5.6)	1 (5.6)	18 (69.2)	**0.673**
	P+ve	7 (87.5)	0 (0.0)	1 (12.5)	8 (30.80	
Total		**23 (88.5)**	**1 (3.8)**	**2 (7.7)**	**26 (100)**	

The investigated risk factors have no significant effect (p>0.05) on the distribution of the antibody titration (table 5). However, the highest level of antibody titration (1:32 and 1:64) was recorded in HIV-positive patients with low haemoglobin concentration. These patients include two males and one married female (table 5).

Table 5. Sero-prevalence and level of anti-*T. gondii* antibodies using LAT.

Factor		N Tested	*P+ve (%)	Distribution of specific antibody titers to *T. gondii* positive reaction (%)			N-ve (%)	p value
				1:16	1:32	1:64		
Sex	Male	42	14 (33.3)	12 (52.2)	0 (0.0)	2 (100)	28 (66.7)	0.234
	Female	40	12 (30.0)	11 (47.8)	1 (100)	0 (0.0)	28 (70.0)	
Age	<40 yrs	49	13 (26.5)	11 (47.8)	1 (100)	1 (50.0)	36 (73.5)	0.593

	≥40 yrs	33	13 (39.4)	12 (52.2)	0 (0.0)	1 (50.0)	20 (60.6)	
Marriage	Yes	61	21 (34.4)	19 (82.6)	1 (100)	1 (50.0)	40 (65.6)	0.471
	No	21	5 (23.8)	4 (17.4)	0 (0.0)	1 (50.0)	16 (76.2)	
*HIV/AIDS	P+ve	50	20 (40.0)	17 (73.9)	**1 (100)**	**2 (100)**	30 (60.0)	**0.601**
	N-ve	32	6 (18.8)	6 (26.1)	0 (0.0)	0 (0.0)	26 (81.3)	
Abortion	Yes	11	3 (27.3)	3 (33.3)	0 (0.0)	0 (0.0)	8 (72.7)	0.490
	No	22	7 (31.8)	6 (66.7)	1 (100)	0 (0.0)	15 (68.2)	
*Hb (g/dl)	<11	42	18 (42.9)	15 (65.2)	1 (100)	2 (100)	24 (57.1)	0.471
	≥11	38	8 (21.1)	8 (34.8)	0 (0.0)	0 (0.0)	30 (78.9)	
TWBC	3000-6999	62	21 (33.9)	19 (82.6)	1 (100)	1 (50.0)	41 (66.1)	0.471
	≥7000	13	5 (38.5)	4 (17.4)	0 (0.0)	1 (50.0)	8 (61.5)	
Total		**82**	**26 (31.7)**	**23 (88.5)**	**1 (3.8)**	**2 (7.7)**	**56 (68.3)**	

*Significant at ($p \leq 0.05$).

As presented in table (6), the investigated risk factors have no significant effect ($p>0.05$) on the rates (IgM Index)of anti-*T. gondii*IgM antibodies. Among the eighteen anti-*T. gondii* IgM seropositive samples, young adults (<40 yrs), marriage, women with no abortion and HIV-negative persons revealed more prevalence rate and more level (IgM Index)of IgM antibody against *T. gondii* without statistically ($p>0.05$) significant differences (table 6). Females recorded more prevalence rate of IgM antibody, but the IgM index was higher in males with insignificant differences ($p>0.05$).

Table 6: Seroprevalence and level of anti-*T. gondii* IgM in people from Sudan.

		N P+ve	IgM Index (%)		P value
			1.1-2	2.1-17	
Sex	Male	8 (44.4)	5 (38.5)	3 (60.0)	0.410
	Female	10 (55.6)	8 (61.5)	**2 (40.0)**	
Marriage	No	6 (33.3)	4 (30.8)	**2 (40.0)**	0.710
	Yes	12 (66.7)	9 (69.2)	3 (60.0)	
Abortion	No	7 (77.8)	5 (71.4)	2 (100)	0.391
	Yes	2 (22.2)	2 (28.6)	**0 (0.0)**	
Age	<40 yrs	11 (61.1)	8 (61.5)	3 (60.0)	0.952
	≥40 yrs	7 (38.9)	5 (38.5)	2 (40.0)	
HIV/AIDS	N-ve	12 (66.7)	9 (69.2)	**3 (60.0)**	0.710
	P+ve	6 (33.3)	4 (30.8)	2 (40.0)	
Total		**18**	**13 (72.2)**	**5 (27.8)**	

About 44.3% of the married people were seropositive for *T. gondii* antibodies (table 7). Three (27.3%) out of the eleven married women in this study were found to be seropositive for *T. gondii* infection. Two (66.7%) of them have had history of repeated abortion (2-3 times), without statistically significant differences (p=0.19) when compared to history of single abortion. Repeated abortion has no statistically significant effect on the *T. gondii* LAT seropositivity (p=0.190) or ELISA IgM seropositivity (p=0.685).

The univariate analysis showed that, the risk factors that significantly associated with both *T. gondii* LAT and ELISA IgM seropositive were AIDS and Hb concentration (p=0.044, 0.007 and 0.038) respectively (Table 7).

Twenty-one of the 50 HIV-positive patients had antibodies to *T. gondii,* and the overall prevalence in this group was 42.0%.Fifteen of the 32 HIV-negative samples were seropositive. An overall prevalence of 46.9% in this group was calculated. IgM was detected in 6 (12.0%) of HIV-positive patients and 12 (37.5%)of HIV-negative samples. The seropositivity of the two groups (HIV-positive and HIV-negative) varies significantly when both LAT (p=0.044) and ELISA-IgM (p=0.007) were used (Table 7). Haemoglobin concentration showed significant effect (p=0.038) on *T. gondii* LAT seropositivity (table 7).

Table 7: Results of univariate association of Risk factors with *T. gondii*seropositivity in people from the Sudan using Chi square.

Risk Factor		N	**Over all +ve**		**LAT +ve**		**ELISA IgM +ve**	
			P+ve (%)	**P value**	**P+ve (%)**	**P value**	**P+ve (%)**	**P value**
Sex	Male	42	20 (47.6)	0.487	14 (33.3)	0.746	8 (19.0)	0.515
	Female	40	16 (40.0)		12 (30.0)		10 (25.0)	
Age	<40 yrs	49	18 (36.7)	0.111	13 (26.5)	0.220	11 (22.4)	0.894
	≥40 yrs	33	18 (54.5)		13 (39.4)		7 (21.2)	
Marriage	Yes	61	27 (44.3)	0.911	21 (34.4)	0.367	12 (19.7)	0.395
	No	21	9 (42.9)		5 (23.8)		6 (28.6)	
HIV/AIDS	P+ve	50	21(42.0)	0.664	20 (40.0)	**0.044**	6 (12.0)	**0.007**
	N-ve	32	15 (46.9)		6 (18.8)		12 (37.5)	

Abortion	Yes	11	3 (27.3)	0.314	3 (27.3)	0.789	2 (18.2)	0.407
	No	22	10 (45.5)		7 (31.8)		7 (31.8)	
Hb (g/dl)	<11	42	20 (47.6)	0.463	18 (42.9)	**0.038**	8 (19.0)	0.613
	≥11	38	15 (39.5)		8 (21.1)		9 (23.7)	
TWBC	<3000	5	1 (20.0)	0.467	0 (0.0)	0.263	1 (20.0)	0.845
	3000-6999	62	29 (46.8)		21 (33.9)		14 (22.6)	
	≥7000	13	5 (38.5)		5 (38.5)		2 (15.40	

The multivariate analysis revealed no significant association between the retested risk factors (Age, HIV/AIDS and Haemoglobin concentration) with *T. gondii* LAT seropositivity ($p>0.05$). However, increasing odds ratios were recorded for the effects of these factors (Age ≥40years, HIV-positive and low haemoglobin concentration) on *T. gondii* LAT seropositivityin the tested people (table 8). The multivariate analysis of the retested risk factors (Marriage and HIV/AIDS) with *T. gondii*ELISA-IgM seropositivity revealed highly significant association between HIV-negative persons and anti-*T. gondii* IgM antibodies seropositivity (table 9).

Table 8: Results of multivariate association of Risk factor with LAT toxoplasma seropositivity in people from the Khartoum state using Chi square.

Risk factors		No of people examined	No of P+ve (%)	Wald (L.R)	p-value	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Age	<40 yrs	49	13 (26.5)	Reference				
	≥40 yrs	33	13 (39.4)	1.094	0.296	1.693	0.631	4.537
HIV/AIDS	N-ve	32	6 (18.8)	Reference				
	P+ve	50	20 (40.0)	1.080	0.299	1.837	0.583	5.786
Hb (g/dl)	≥11	38	8 (21.1)	Reference				
	<11	42	18 (42.9)	2.260	0.133	2.262	0.780	6.559

Table 9: Results of Multivariate Association of Risk factor with ELISA IgM toxoplasma seropositivity in people from the Khartoum state using Chi square.

Risk factors		No of people examined	No of P+ve (%)	Wald (L.R)	p-value	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Marriage	Yes	61	12 (19.7)	Reference				
	No	21	6 (28.6)	0.342	0.559	1.429	0.432	4.731
HIV/AIDS	P+ve	50	6 (12.0)	Reference				
	N-ve	32	12 (37.5)	6.495	**0.011**	4.280	1.399	13.094

Discussion

Human toxoplasmosis has been well studied worldwide. The estimated seroprevalence of 43.9% in the present study is comparable to many other reports from exposed group in Africa (29 - 31). Although the difference was not significant, the overall *T. gondii* seroprevalence was higher in males than females, older than younger, married than single and women with no history of abortion than those with history of abortion. Similar findings concerning age (31 – 32) and sex (33 - 38) were reported in Tanzania and Brazil respectively. Our result concerning history of abortion was disagreeing with that of Khalil *et al.* who reported significant association between toxoplasmosis and abortion (27). In agreement with Gongora-Biachi *et al.* (1998), the overall *T. gondii* seroprevalence was higher in HIV-negative than HIV-positive subjects (36). However, Khalil *et al.* reported higher prevalence rate in HIV-positive people (27). The differences may be due to the different serological test and antibody titration used as cut-off point. Moreover, our HIV-patients were under continuous follow up for prevention of opportunistic infections such as toxoplasmosis. Additionally, the overall seroprevalence of toxoplasmosis appear lower in the HIV-positive patients, because anti-*T. gondii* IgM antibodies was more frequently detected in HIV-negative individuals than the HIV-positive patients. This point was clearly justified when HIV-positive revealed significantly

(p=0.044) higher LAT-seropositivity, while HIV-negative revealed significantly (p=0.007) higher ELISA-IgM seropositivity in this study. This is not surprising because the immune response of the HIV-positive patient is affected. Increasing odds ratios without significant association (p>0.05) were recorded for Age ≥40 years, HIV-positive and low haemoglobin concentration inthe multivariate analysis of risk factors associated with Toxoplasma-LAT seropositivity. However, the multivariate analysis showed highly significant association between HIV-negative persons and anti-*T. gondii* IgM antibodies seropositivity. These findings prefer the use of screening tests or IgG detection tests for HIV-patient. Antibody titers were higher in HIV infected persons than in those who were uninfected. Our present findings support (on the need for special attention to anti-*Toxoplasma gondii* antibodies during HIVcare (40 - 41). Generally, the growing AIDS epidemic is a disturbing reminder that opportunistic infections such as toxoplasmosis remain a major potential threat to human health in the Sudan.

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