Research Article

Available Online at: www.ijphr.com

International Journal of Pharmaceuticals and Health care Research

ISSN: - 2306 – 6091

SEROPREVALENCE OF *TOXOPLASMA GONDII* AND ASSOCIATED RISK FACTORS AMONG BLOOD DONORS AT GONDAR UNIVERSITY HOSPITAL, NORTHWEST ETHIOPIA

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Abstract

Transmission of *Toxoplasma gondii* by transfusion of blood from asymptomatic individuals is possible. Despite this reality, the epidemiology of *T. gondii* infection among blood donors in Ethiopia is not well studied. Therefore, the aim of this study was to determine seroprevalence and associated risk factors of toxoplasmosis among blood donors attending Gondar University Hospital, Northwest Ethiopia. A cross-sectional study was conducted from February to April 2012 among blood donors attending Gondar University Hospital Blood Bank. A total of 422 of blood donors were interviewed about sociodemographic characteristics and risk factors for *Toxoplasma gondii* infection. The blood samples were collected and assayed for *Toxoplasma gondii* antibody using latex agglutination test. One hundred and seventy one (40.5%) of the study participants was positive for anti-*T. gondii* antibodies. From the positive blood donors for *T. gondii*, 38.7% (135/351) were males and 50.7 % (36/71) were females respectively. From different age groups, the positivity rate was highest (47.6%) among age category of 49-64 years. Among the different risk factors assessed, eating uncooked meat (AOR=1.291, 95%CI=1.092-3.578), handling cat excreta (AOR=5.683, 95%CI=3.390-9.527); eating raw vegetables and fruits (AOR=2.276, 95%CI=1.309-3.957) were statistically significantly associated with *T. gondii* seropositivity. The high seroprevalence of *Toxoplasma gondi* among healthy blood donors alarm the need to screen blood donors for Toxoplasmosis prior to transfusion even though extensive study on the rate of the transmission of the parasite by blood transfusion is essential to conclude.

Keywords: *Toxoplama gondii*, Blood donors, Gondar University Hospital, Ethiopia.

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Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite and is distributed globally. It was estimated that one-third of the world's human population is exposed to the threat of this parasite. *T. gondii* is an opportunistic pathogen, which is generally asymptomatic in the immunocompetent individual[1-3]. Toxoplasmosis is a major public health problem, with a high socio economic impact in terms of human suffering including the cost of caring for sick, mentally retarded and blind children. The parasite is an extremely successful pathogen, responsible for significant morbidity and mortality. Toxoplasmosis most commonly manifests as a mild, flu-like illness with low grade fever, myalgia, malaise and headache[4-6].

The most important channels for transmission to humans are by ingestion of food or water contaminated with oocysts shed by cats, by eating undercooked or raw meat containing infective tissue cysts and via transplacental transfer, notably when the mother becomes infected for the first time during pregnancy. Human infection with *T. gondii* is a huge challenge for which there is no effective treatment[7-8]. In addition to the above mentioned route of transmissions, the possibility of transmission of toxoplasmosis via blood transfusion was suggested by different scientists. The parasite can be transmitted through blood or leucocytes from infected donors. In humans parasitemia caused by this organism has been demonstrated in asymptomatic blood donors [6]. Since the advent of the HIV/AIDS pandemic, concurrent *toxoplasma* infection has become an important health problem in the world. The emergence of the disease is an urgent health problem requiring proper management, but data concerning its prevalence, pleomorphic clinical manifestations, outcome of treatment and disease prevention are not available[9]. The majority of infections are asymptomatic and patients rarely experience symptoms or complications which can make detection and control of *T. gondii* transmission challenging. This is further complicated by the fact that *T. gondii* leads to significant clinical consequences given their infectious nature, chronicity and therapeutic difficulties. Hence, serologic screening will allow early detection of *T. gondii* infection in asymptomatic carriers and prevention of adverse sequelae in blood recipients[10]. *T. gondii* seropositivity rates in blood donor and general population studies vary considerably around the world. *T.gondii* seropositivity was reported in different parts of the world. In Ethiopia, there was a survey which has been undertaken before nine years among six different geographical regions and a seroprevalence of 74.4% was obtained [11]. But the current prevalence of *T. gondii* and the relative contribution of the various routes of transmission in humans in Ethiopia have not been adequately studied. Therefore, this study was designed to determine the seroprevalence of toxoplasmosis and associated risk factors among blood donors in Gondar, northwest Ethiopia.

Materials and Methods

Study area and design

A cross-sectional study was conducted from February to April 2012 at Gondar University Hospital. Based on the 2007 census result, Gondar has a total population of 207,044 [12]. The hospital is the largest hospital in the region serving as a teaching hospital for more than 5 million people of the region. The data was obtained from Gondar University Hospital Blood Bank; this unit provides blood for patients requiring blood transfusion for different reasons in the hospital and averagely about 10 units of blood per day can be collected. The blood was collected by an experienced nurse working in the unit and about 450 ml of blood from any adult blood donor was collected.

Sample size and sampling techniques

The sample size for this study was calculated by using single proportion formula at 95% confidence interval (CI) level (Z (1-ά/2) = 1.96). An expected prevalence of 50% was taken since there was no study conducted regarding this topic in the area and 5% marginal error was also used. Then the sample size was calculated as n = [Z 1- a/2] 2 P (1-p]/d2, where: n = sample size, P = proportion problem in the study area, Z 1-a/2 = CI of 95%, d = marginal error to be tolerated. By adding 10% of contingency, 422 blood donors were included in our study. Blood donors who were HIV, HCV, HBs Ag and *Treponema pallidum* seronegatives and those aged 18–64 years were selected to participate in the study. These blood donors who visited the blood bank were enrolled consequently until the required sample size achieved.

Data collection and laboratory analysis

A structured questionnaire was used to collect socio-demographic data and associated risk factors like presence of cat in the house, habit of handling cat excreta, presence of dog at home, raw or uncooked meat consumption, washing hand before eating, consumption of raw or uncooked vegetable and fruit, type of blood donors, previous history of donation and previous history of transfusion.

Five ml venous blood was collected, centrifuged and eventually serologically tested for detection of *T. gondii* antibody. The test was done using serological latex agglutination test kit (Toxo-Spain, Joaquim costa, 182 planta, 08390 Montgat, Barcelona, Spain). Toxo-latex test is a rapid slide agglutination procedure, developed for the direct detection of anti-toxoplasma antibodies in human serum. The assay was performed by mixing suspension of latex particles coated with antigenic extract of *T.gondii* with the test samples. The presence a visible agglutination indicates the presence of anti-Toxoplasma antibodies in the sample. And the absence of a visible agglutination indicates the absence of the antibody.

Quality control

After data collection process, the data were checked for completeness and any incomplete or misfiled questionnaires were checked. Then the result of laboratory examination was recorded on well-prepared format carefully and finally it was attached with the questionnaire. Within two hours' of specimen collection, the collected sample was processed in the laboratory by strictly following Standard Operational Procedure (SOP).

The appropriateness of the kit was checked using the manufacturer's instruction and also positive and negative controls of the kit was used in each batch of the test in order to assure the quality of the results.

Data analysis

Data were double entered and analyzed by using SPSS 20 database software programme. Descriptive statistics were used to give a clear picture of the background variables like age, sex and other variables from well-structured questionnaire. The frequency distribution of both dependent and independent variables were worked out. The association between the independent and dependent variables were measured and tested using Odds Ratio (OR) and 95% confidence interval (CI). The relative contribution of each selected variables to the outcome of interest were assessed using logistic regression.

Ethical clearance

Ethical clearance was obtained from Ethical Clearance Committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences University of Gondar. Additionally, after explaining the importance, purpose and procedure of the study briefly, an informed written consent was obtained from study participants. Anyone not willing to take part in the study had full right to not participate in the study and/ or the study participants had a right to be withdrawn from the study at any time of the study duration and confidentiality of the study participants was also maintained.

Results

Sociodemographic characteristics of study participants

From 422 individuals that were involved in this study, 351(83.2%) of the respondents were males and 71(16.8%) were females. The mean age of the respondents was 28 years and 76.5% were at the age between 18-32 years. Out of all, 64 % were urban resident. Regarding their educational status, the majority of respondents 229 (54.3%) were students at high school and above that (Table 1).

Prevalence of *T. gondii*

Among 422 study subjects, 171(40.5%) were positive for *T.gondii* antibody. From all the blood donors that were positive for *T .gondi*, 38.5% (135/351) and 50.7 % (36/71) were males and females respectively. From different age groups the positivity rate was highest (76.5%) among age category of 18-32 years. The distribution of seropositivity among blood donors in relation to their occupation indicated that 36.4%, 37.8%, 41.9%, 55.5% and 85.9% were government employer, farmer, merchant, unemployed and daily laborers respectively (Table 1).

Risk factors for Toxoplasmosis

The risk factors associated with seropositivity of toxoplasmosis on crude Bivairate analysis were: presence of cat at home, habit of handling cat excreta, habit of eating uncooked meat, habit of eating raw vegetable and fruits. Individuals who

had cat in their homes were found two times (COR=2.266, 95%CI= 1.518, 3.383, p-value <0.00001) more likely to be sero positive for toxoplasmosis than those who did not have cat in their home and individuals who handle their cat excreta found almost six times (COR=5.840, 95% CI=3.573- 9.544, p-value<0.00001) more likely to be sero positive for toxoplasmosis than those who do not handle cat excreta.

Individuals who had habit of eating uncooked meat were found two times (COR=2.094, 95% CI= 1.406-3.117, p-value<0.00001) and individuals who had habit of eating uncooked vegetables and fruits were found three times (COR=2.941, 95% CI= 1.744-4.959, p-value<0.00001) more likely to be sero positive for toxoplasmosis than those who did not eat (Table 2). However, regarding other characteristics like sex, age group, educational status, occupation, residence, presence of dog at home, previous history of transfusion and type of donation did not show any statistical association with toxoplasmosis positivity (Table 2).

Among the risk factors that are found to be significantly associated in the Bivairate analysis, only habit of handling cat excreta, habit of eating uncooked meat and habit of eating raw vegetables and fruits were found to be significantly associated with *T. gondii* positivity in multiple logistic regression analysis. Those blood donors who had a habit handling cat excreta found almost 5.6 times (AOR=5.683, 95%CI= 3.390, 9.527) more likely to be sero positive than those who did not handle cat excreta and blood donors who eat uncooked meat found almost 1.3 times (AOR=1.291, 95%CI= 1.092, 3.578) more likely to be sero positive than those who do not eat.

Those blood donors who had a habit of eating uncooked raw vegetables and fruits were almost 2 times (AOR=2.276, 95%CI= 1.309, 3.957) more likely to be sero positive than those who did not have the habit (Table 2).

Discussion

Currently, therapy as well as vaccine is not fully effective against *T.gondii* infection. This fact strengthens that an effort to reduce transmission of the diseases is very crucial to lower the burdon of the disease. Therefore a cross sectional study to determine the prevalence of antibodies against *T.gondii* and its associated risks factor were carried out in northwest Ethiopia and a prevalence rate of 40.5% was found.

The findings of this study was in agreement with previous study conducted in Eastern Saudi Arabia 40% [13], in Netherlands 40.5%[14], and with only little difference with a study done in London 36% [15], However the present study showed much lower prevalence than that was reported in Bahir Dar, northwest Ethiopia, 70.29% [27], Brazil 75% [16], south western Saudi Arabia 52.1%[17], Egypt 59.6% [18], Kenya 54% [19]. On the other hand, prevalence of *T.gondii* seropositivity in the present study was higher than those studies that are conducted in Turkey 20 % [20], Chile, 20.3-21.2 [21], India 20.3 % [22], in Durango Mexico 6.1% and 9% in two blood banks[23]. The most plausible explanation for such differences is might be because of the difference in socio economical and/or cultural difference; in addition to these, using different type of laboratory tests in the different studies could be the probable reasons.

Association between risk factors and *T.gondii* seropositivity were analyzed. Statistical analysis showed that seropositivity of *T.gondii* had not statistical difference between men and women (P value = 0.057). This is similar with a study done in Egypt [18]. The small number of women participated in this study can be explained by the feature of Ethiopian blood donation trends which mainly involves male family members.

Regarding to the other risk factors for *T.gondii* infection, habit of the handling cat excreta, eating of undercooked meat, raw vegetables and fruits, had shown to have statistically significant association with *T.gondii* infection among the blood donors. Among these, handling cat excreta as one of the determinant factors for the acquisition of toxoplasmosis, had showed greatest degree of association with *T.gondii* infection (AOR=5.683, 95% CI=3.390-9.527) and this might be due to ingestion of oocytes in contaminated food and water by those who had a habit of handling cat excreta and poor hygienic practice.

The finding of this study also revealed that eating uncooked meat had strong association with *T.gondii* seropositivity (AOR=1.291, 95% CI= 1.092, 3.578). This finding was similar to previous

studies done in Jalisco Mexico and Northern Mexico Durango city[24-25]. This might be due to the fact that infection in humans generally occurs either by ingesting viable tissue cysts in raw or undercooked meat or by ingesting oocysts shed in the feces of a cat. However, this finding was not supported by another study done in India where the authors reported no association between *T.gondii* infection and eating uncooked meat[23].Such differences might be because of the difference in the health condition of the cattles among the two countries.

The present study also identified that eating raw vegetables and fruits as one of the significantly associated risk factor to harbor *T.gondii* infection (AOR=2.276, 95% CI=1.309-3.957) among the blood donors. Blood donors who had a habit of eating raw vegetables and fruits were more likely to have *T.gondii* infection than those blood donors who had not a habit of eating raw vegetables and fruits. This may be due to the fact that habit of eating raw vegetables and fruits increases the chances of ingesting oocytes and acquiring *T.gondii* infection.

Generally, the present study showed that *T.gondii* is highly prevalent among blood donors in Gondar, northwest Ethiopia. Our findings reflected the occurrence of high potential of transmitting the infection to a patient who needs blood during blood transfusion in the study area since it is well known that the parasite can survive in citrated blood for 28 days at 4^0c, thus refrigeration of blood units during storage cannot prevent the transmission of the parasite[26]. In conclusion, our findings showed a high seroprevalence of *T. gondii* among blood donors in Gondar. Habit of handling cat excreta, consumption of undercooked meat and raw vegetables and fruits were the major risk factors in the transmission of the diseases. It may be appropriate to include screening of blood for *T. gondii* among the pre transfusion blood tests even though extensive study on the rate of the transmission of the parasite by blood transfusion is essential to conclude. Especially, such kinds of screening tests should be carried out on blood to be transfused to immune compromised patients and pregnant women.

The strength of the present study is that standard serological technique to detect antibody against *T.gondii* was used. Qualified medical laboratory technologists have undertaken the test and standard questionnaire were used. However, the test used in the study cannot differentiate acute from chronic infection and this can be the limitations of the study.

Acknowledgement

We would like to express our sincere and heartfelt gratitude to all staffs of Gondar University Hospital Laboratory, especially the Serology Laboratory for their technical assistance throughout the research work and all staffs of the Blood Bank of the hospital for their kind cooperation during sample collection. We thank all blood donors who have participated in this study. We also thank Amhara Regional Health Bureau for covering the budget for this study

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Table No. 01: Socio-demographic characteristics and seroprevalence of *Toxoplasma gondii* of blood donors attending Gondar University Hospital Blood Bank, Gondar, Northwest Ethiopia, 2012

Variables		Frequency (No)	Percentage (%)	Negatives (No, %)	Positives (No, %)
Sex	Male	351	83.2	216(61.5)	135(38.5)
	Female	71	16.8	35(49.3%)	36(50.7%)
Age groups	18-32yrs	323	76.5	184(57%)	139(43%)
	33-48yrs	78	18.5	56(71.8%)	22(28.2%)
	49-64yrs	21	5	11(52.4%)	10(47.6%)
Residence	Urban	270	64	154(57.1%)	116(42.9%)
	Rural	152	36	97(63.8%)	55(36.2%)
Educational status					
	Illiterate	87	20.6	52(59.8%)	35(40.2%)
	Primary education	106	25.1	63(59.5%)	43(40.5%)
	High school students and above	229	54.3	136(59.4%)	93(40.6%)
Occupation					
	Gov't employed	44	10.4	28(63.6%)	16(36.4%)
	Farmer	130	30.8	81(62.2%)	49(37.8%)
	Merchant	31	7.3	18(58.1%)	13(41.9%)
	Unemployed	18	4.3	8(44.5%)	10(55.5%)
	Daily Laborers	199	47.2	116(14.1%)	83(85.9%)
Total				251(59.5%)	171(40.5%)

The appropriate position of this table (table 1) on the text is on page 8 after the second paragraph.

Table No. 02: Bivairate and multivariate logistic regression of selected variables to *Toxoplasma gondii* seropositivity among blood donors attending Gondar University Hospital Blood Bank, Gondar, Northwest Ethiopia, 2012

Variables		Negative	Positive	COR (95% CI)	p-value	AOD (95% CI)	p-value
Sex	Male	216	135	1*		-	-
	Female	35	36	1.646(.986-2.748)	0.057		
Age groups	18-32yrs	184	139	1*			
	33-48yrs	56	22	0.520(0.303-0.892)	0.018	-	-
	49-64yrs	11	10	1.203(0.497-2.914)	0.682		
Residence	Urban	154	116	1.328(0.882-2.000)	0.174	-	-
	Rural	97	55	1*			
Educational status	Illiterate	52	35	0.984(0.595-1.628)		-	-
	Primary education	63	43	0.998(0.625-1.595)	0.951		
	Secondary education and above	136	93	1*	0.994		
Occupation	Gov't employed	28	16	1*			
	Farmer	81	49	1.059(0.521-2.152)	0.875		
	Merchant	18	13	1.264(0.493-3.240)	0.626	-	-
	Unemployed	8	10	2.187(0.718-6.666)	0.169		
	Daily Laborers	116	83	1.252(0.637-2.461)	0.514		
Presence of cat in the house	Yes	80	88	2.266(1.518-3.383)**	<0.00001		
	No	171	83	1*			
Habit of handling cat excreta	Yes	29	74	5.840(3.573-9.544)**	<0.00001	5.683(3.390-9.527)**	<0.001
	No	222	97	1*		1*	
Presence of dog in home	Yes	85	52	0.853(0.562-1.296)	0.457		
	No	166	119	1*			

Habit of eating uncooked meat	Yes	113	108	2.094(1.406-3.117)**	<0.00001	1.291(1.092-3.578)**	0.003
	No	138	63	1*		1*	
Habit eating uncooked vegetable and fruits	Yes	175	149	2.941(1.744-4.959)**	<0.00001	2.276(1.309-3.957)**	0.004
	No	76	22	1*		1*	
Habit of washing hand before eating	Yes	246	169	1*	0.521	-	-
	No	5	2	0.582(0.112-3.036)			
History of previous transfusion of blood	Yes	1	0	0	1.000	-	-
	No	250	171	1*			
Type of donation	Voluntary	55	41	1*	0.620	-	-
	Replacement	196	130	0.890(0.561-1.411)			

The appropriate position of this table (table 2) on the text is at the end of the result.