Sereoprevalence of Immunoglobulin-G and of Immunoglobulin-M *Anti-Toxoplasma gondii* Antibodies in Human Immunodeficiency Virus Infection/Acquired Immunodeficiency Syndrome Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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Abstract

Background: In Ethiopia, only a few studies on the seroprevalence of toxoplasmosis have been carried out among HIV/AIDS patients. The objective of this study was to determine the seroprevalence of toxoplasmosis among HIV/AIDS patients at Tikur Anbessa Specialized Hospital, Ethiopia, and to determine risk factors associated with seroprevalence.

Methods: Blood samples were collected from randomly selected 150 HIV-positive patients and IgM and IgG anti-toxoplasma antibodies were quantified by using Enzyme immunoassay technique (HUMAN-ELISA, GERMANY). Ethical approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine, Addis Ababa University. Questionnaire was administered to assess the risk factors associated with the prevalence of toxoplasmosis in HIV patients.

Results: Of the one hundred fifty patients 108 (72%) were females and 42 (28%) were males. The mean (sd) age was 38.4 (9.5). Based on IgG anti-Toxoplasma antibodies status, the seroprevalence of toxoplasmosis in HIV-positive patients was 94%. No IgM antibody was detected. Consumption of raw vegetables and not having primary information about toxoplasmosis were significant association with the presence of anti-Toxoplasma antibody (p<0.05). Inexact logistic regression analysis consumption of raw vegetable (adjusted OR=7.49, 95% CI 1.29-58.93) was significant risk factor for toxoplasmosis and having information about toxoplasmosis (adjusted OR=.083,95% CI .011-.499) had significant protective effect. The mean (sd) CD4 count was 341.1 (173.6) cells/µl. The association between the presence of anti-toxoplasma antibody and CD4+ T lymphocyte cells count was not statistically significant.

Conclusions: The findings showed that there is a high prevalence of chronic toxoplasmosis in HIV/AIDS patients and the risk factors were consumption of raw vegetable and lack of information about toxoplasmosis. Therefore, routine screening for Toxoplasma should be undertaken for all HIV-infected patients. Moreover, creating awareness about toxoplasmosis and its risk factors should be prioritized.

Background

Toxoplasmosis is caused by infection with the obligate, intracellular parasite, *Toxoplasma gondii* [1]. It readily infects human beings and clinical illness is relatively uncommon [2]. *Toxoplasma gondii* is estimated to affect around two billion people worldwide, and it has gradually evolved over the pass decades to be among the most common opportunistic diseases in Human Immunodeficiency Virus infection/Acquired Immunodeficiency Syndrome (HIV/AIDS) in developing countries [3]. Transmission of the disease mainly occur through ingestion of tissue cysts in raw and undercooked meat, exposure to oocysts in contaminated vegetable or contact with faeces of a felid. In Ethiopia, general prevalence is about 80% in the adult population [2], and 93.3% among HIV-infected patients seeking either HIV or immunological testing at St. Paul's Hospital, Addis Ababa, Ethiopia [4]. Disease due to infection with the protozoan parasite, *Toxoplasma gondii,* is a major complication of HIV-induced immunosuppression [5], and responsible for congenital infections and abortion in immunocompetent individuals individuals and opportunistic infections in immunodeficient individuals [1]. Toxoplasmosis in patients with AIDS is usually the result of reactivation of latent infection. Toxoplasmosis among Acquired Immunodeficiency Syndrome (AIDS) patients manifests primarily as a life threatening condition, toxoplasmic encephalitis (TE) [4]. Toxoplasmic encephalitis usually occurs in HIV–infected patients with CD4+ T- cell counts <100/µL [6]. In Ethiopia only a few studies on the seroprevalence of toxoplasmosis have been carried out among HIV/AIDS patients. The objective of this study was to determine the seroprevalence of toxoplasmosis among HIV/AIDS patients at Tikur Anbessa Specialized Hospital and to determine risk factors associated with seropositivity.

Methods

Description of the study design and setting

A cross-sectional study was carried out from October, 2011-February, 2012 at Tikur Anbessa Specialized Hospital at Provider Initiated Testing and Counseling (PITC) sub unit under the Infectious Disease Unit to determine the prevalence of toxoplasmosis in HIV/AIDS patients visiting the hospital. Tikur Anbessa Specialized Hospital, which is located in Lideta sub city in Addis Ababa,

the hospital works under Addis Ababa University. It provides multidisciplinary clinical service for all patients who come from different parts of the country and the Hospital started antiretroviral treatment service, since March 2005.

Recruitment of study participants

150 HIV/AIDS patients were randomly selected from the provider initiated testing and counseling (PITC) unit to participate in the study and eight individuals were not volunteer to participate in the study. Using simple random sampling technique, patient's history card was selected in the morning and afternoon for twenty consecutive working days. Moreover, on average twenty one patients visited PITC unit daily during the study period.

Ethical consideration

Ethical approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine, Addis Ababa University (Ref. No. 079/11/Ext.). After obtaining written/verbal consent from 150 randomly selected HIV/AIDS patients, relevant information was obtained using a questionnaire. Moreover, 5 ml of venous blood was collected from each patient and the collected sera were kept at -20°C, until use.

Sample analysis

IgM and IgG antibodies to *Toxoplasma gondii* from serum samples were quantified by an Enzyme immunoassay technique, according to the manufacturer's instructions (HUMAN- ELISA, GERMANY) and CD4+ T lymphocyte cell counts less than a month's period were taken from registration book of Tikur Anbessa Specialized Hospital.

Data management and analysis

Data obtained from questionnaire and laboratory tests were entered using Microsoft Excel 2010, and analysis was carried out using STATA version 11 software and SPSS version 20. The Fisher's exact test was used for testing the association between possible risk factors and disease transmission, also for the association between CD4+ T lymphocyte cell counts and *Toxoplasma* seropositivity. The Alpha (α) value, allowance for type one error, was set at 0.05, and therefore, a p-value of less than 0.05 was regarded as statistically significant to reject the null hypothesis. Odds ratio and corresponding 95% Confidence Interval (CI) were used to quantify the degree of associated with different variables. The relative contribution of each selected variables to the outcome of interest were also assessed using exact logistic regression model.

Results

Socio-demographic characteristics

The selected 150 study subjects were included in the questionnaire survey and different socio demographic characteristics were considered for the study (Table 1).

Risk factors associated with seroprevalence of *Toxoplasma gondii*

Results from bivariate analysis: Risk factors associated with the seroprevalence of *Toxoplasma gondii* like living with cat, consumption of uncooked meat and raw vegetables and primary information about toxoplasmosis were assessed. Of these, primary information about toxoplasmosis (P<0.05) and consumption of raw vegetable (P<0.05) were significantly associated with the seroprevalence of the disease (Table 2).

Results from exact logistic regression: Different risk factors were assessed before and after adjusting seemingly significant predictor in the exact logistic regression model. The like hood of experiencing

Socio demographic Characteristics		IgG antibody				
		Total N	Positive N (%)	Fisher's Exact	Crude odds ratio (95% CI)	p-value
Age	11-20	2	2 (100%)	0.912	1.213(.593- 2.743)	0.729
	21-30	36	34 (94.4)			
	31-40	67	62 (92.5)			
	41-50	26	24 (92.3)			
	51-60	16	16 (100%)			
	61-70	3	3 (100%)			
Sex	Female	108	100 (92.6)	0.446	3.26 (.414 -149)	0.450
	Male	42	41 (97.6)			
Marital status	Married	126	118 (93.7)	0.189	392 (.077- 2.60)	0.377
	single	24	23 (95.8)			
Level of education	Illiterate	29	27 (19.15)	0.115	2.30 (.921-7.60)	0.080
	Primary education	64	60 (42.55)			
	Secondary education and above	57	54 (38.30)			
Religion	Orthodox	130	123 (94.6)	0.343	620 (.253- 1.97)	0.4309
	Muslim	6	6 (100)			
	Protestant	14	12 (85.7)			
Income (in Birr)	<300	76	71 (93.4)	0.746	1.49 (.580- 4.64)	0.518
	300-600	39	37 (94.9)			
	>600	35	33 (94.3)			
Highly Active Antiretroviral Therapy (HAAR)	With HAART	144	136 (94.4)	1.000	298 (.027-15.63)	0.629
	Without HAART	6	5 (83.3)			

Table 1: Socio-demographic characteristics of *HIV/AIDS patients with and without* Toxoplasma infection using Fisher's exact and exact logistic regression model.

Risk factors		IgG antibody		
		Total N	Positive N (%)	Fishers exact
Contact ownership				
	Yes	61	57 (93.4)	1.000
	No	87	84 (96.6)	
Consumption of uncooked meat				
	Yes	83	77 (92.3)	0.732
	No	67	64 (95.5)	
Consumption of raw vegetables				
	Yes	102	99 (97.1)	0.030
	No	48	42 (87.5)	
Primary information about toxoplasmosis				
	Yes	21	16 (76.2)	0.0030
	No	129	125 (96.9)	

Table 2: Seroprevalence of toxoplasmosis in relation to various possible risk factors in HIV/AIDS Patients using Fisher's exact test.

Characteristics		Crude odds ratio (COR) (95% Confidence Interval)		Adjusted odds ratio (AOR) (95% Confidence Interval)	p-value
Cat ownership	Yes	1.17 (.223-5.73)	1.000	574 (.079- 3.35)	0.747
	No	1.00			
Consumption of uncooked meat	Yes	1.65 (.337-10.64)	0.729	2.36 (.387-18.97)	0.487
	No	1.00			
Consumption of raw vegetables	Yes	4.65 (.942-30.14)	0.060	7.91 (1.29-65.82)	0.021
	No	1.00			
Primary information about toxoplasmosis	Yes	105 (.018- .542)	0.005	083 (.011-.499)	0.004
	No	1.00			

Table 3: Exact logistic regression analysis on seemingly significant predictors of *T. gondii* infection.

CD4 count (cells/µL)	IgG Antibody		
	Total N	Positive N(%)	Fishers exact
<200	33	30 (90.9)	0.089
200-500	94	91(96.8)	
>500	23	20 (86.9)	

Table 4: Status of antibodies to *Toxoplasma gondii* based on different ranges of CD4+ T cell counts.

Toxoplasma gondii antibodies in their serum was higher among HIV/AIDS patients who consumed raw vegetables, and the prevalence of *T. gondii* was lower among study participants who did have information about toxoplasmosis. Moreover, consumption of raw vegetable (adjusted OR=7.49, 95%CI 1.29-58.93) was significant risk factor for toxoplasmosis and having information about toxoplasmosis (adjusted OR=.083, 95%CI .011- .499) had significant protective effect (Table 3).

Laboratory results

ELISA results were interpreted as IgM and IgG positive and negative in terms of absorbance (optic density). A total of 150 immune compromised (HIV/AIDS) patients participated in this sero-survey. 141 (94%) of the patients were sero-positive anti-*Toxoplasma gondii* IgG antibodies, whereas anti-*Toxoplasma gondii* IgM antibodies were not detected in patients' serum. There was no statistically significant association between CD4+ T lymphocyte cells count and anti-*Toxoplasma gondii* IgG antibodies found (P>0.05) (Table 4).

Discussion

In the current study, the seroprevalence of *Toxoplasma gondii* showed 94% in HIV/AIDS patients, as measured by serum antibody level of IgG, which is comparable with previous study in the same study area, *Toxoplasma* infection in Addis Ababa was observed with prevalence of 93.3% [4]. The seroprevalence of *Toxoplasma gondii* in HIV/AIDS patients in this study differ much from that obtained in other countries: 75.4% in Nigeria, 60% from Cote d'Ivoire, 58.4% in Tunisia, 53.6% in Benin, 40.2% in Senegal [7], and 54% prevalence in HIV positive adults in Uganda (3), 41.2% in HIV/AIDS patients in Malaysia [8]. These variations may be due to difference in geographical area and living condition.

In agreement with the current study, the seroprevalence of *Toxoplasma gondii* estimated for human population varies greatly among different countries, among different geographical areas within the same country, and among different ethnic groups living in the same area [9]. The differences in seropositivities are based on several factors like the geographical distribution of the study sites and socioeconomic status [8].

Recent acquired *Toxoplasma gondii* infection is usually based on the detection of specific IgM antibody. In the current study, no anti-*Toxopalsma gondii* IgM antibodies were detected. In agreement with other similar studies: 1% in Iran [10], 0% in Mexico [11] and 0% in

India [12]. CD4+ T lymphocyte cell count is one of the prognostic marker in HIV/AIDS patients, in this study, there was no statistical significant association between CD4 at different ranges of CD4 cells/µ L (<200, 200-500 and >500) and *Toxoplasma* infection. In agreement with the current study, there was no significance association between CD4 count <200 VS >200 [13]. The majority of the studied population was on antiretroviral treatment regimens. Recently, Highly Active Antiretroviral Therapy (HAART) against HIV infection has been advocated for improving the immune status of patients, thus reducing the incidence of opportunistic infections [14]. With the widespread use of HAART, the incidence of Central Nervous System (CNS) toxoplasmosis has decreased [15], and between 97% and 100% of HIV infected patients with *Toxoplasma* encephalitis have anti-*Toxoplasma gondii* IgG antibodies [16], and low CD4 cell count is a significant factor contributing to the occurrence of active toxoplasmosis in patients with AIDS [17].

Socio demographic characteristics were not significantly associated with the seroprevalence of *Toxoplasma gondii*. Similarly, studies in India demonstrated that there is no association between the prevalence o f toxoplasmosis and demographic and baseline characteristics [12]. No significant relationship was observed between seroprevalence of toxoplasmosis and socio-demographic and baseline characteristics of the patients, the HIV transmission risk factors and the CD4 counts [10].

The current study also showed the likelihood of experiencing *Toxoplasma gondii* antibodies in their serum was significantly higher in patients who consumed raw vegetables and who did not have sufficient information about toxoplasmosis. This is probably due to the living condition and the dietary habit of the people. In Ethiopia, eating raw vegetable is common and treatment of it before eating is uncommon. Increasing the age, raw or half-cooked meat use, keeping cat in house and living in warm, humid and low-revenue places increases the prevalence of toxoplasmosis [18]. In Mexico and Brazil, cats are fed with raw viscera and leftover, increasing the chance of human infection [14].

High seroprevalence of chronic *Toxoplasma* infection among the study population seems reasonable as raw or insufficiently cooked meat prepared in a various favorite cultural food is consumed. In addition, cats are abundant to cause environmental contamination and the climate is favorable to favor survival of the parasite [4]. Cultural values have great impact on the prevalence of a disease, in our culture cats are always in close proximity to the people. One of the causes for this high prevalence is attributed to the close contact of humans with cats [19].

This study showed chronic toxoplasmosis was high in HIV/AIDS patients. Socio-demographic characteristics and different risk factors were also assessed in association with the incidence of toxoplasmosis. Hence, *Toxoplasma gondii* has emerged as an important opportunistic infection in HIV/AIDS patients. So, HIV/AIDS patients should be screened for anti-*Toxoplasma* antibodies and public awareness about the infection should be prioritized.

Competing Interests

The author(s) declare that they have no competing interests.

Author's Contributions

Getachew Tilahun, Daniel Fekade and Nega Berhe conceived of the study, and participated in its design and coordination and helped to draft the manuscript.

Haileluel Aleme carried out the serological studies and drafted the manuscript.

Girmay Medhin participated in the design of the study and performed the statistical analysis. All authors read and approved the final manuscript.

Author's Information

On behalf of the authors, I take the full responsibility for the article during submission and peer review.

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