Original Article

Seroprevalence of IgM and IgG Antibodies to *Toxoplasma* infection in healthy and HIV-positive adults from Northern Nigeria

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Abstract

Introduction: We examined the seroprevalence of toxoplasma infection in HIV-negative and -positive adults from Zaria, Northern Nigeria, and assessed its relationship with demographic, clinical, and immunological findings.

Methodology: In a six-month cross-sectional study undertaken in 2008, sera of 219 adults, including 111 consecutive HIV-infected adults and 108 healthy HIV-negative adult volunteers from Zaria, Northern Nigeria, were examined for IgG and IgM antibodies to *toxoplasma* by ELISA. Clinical characteristics of the HIV-infected patients were documented. Differences in toxoplasma seropositivity between HIV-positive and negative adults were sought. The relationship between toxoplasma seropositivity and variables such as age, sex and antiretroviral (ART) status, as well as HIV clinical staging and CD4 cell counts were also determined. P<0.05 was considered significant.

Results: The seroprevalence of toxoplasma infection (IgG positive and or IgM positive) was 32.4% in HIV-negative healthy adults and 38.7% in HIV-infected adults (P>0.05). The rate of IgM seropositivity was 4.6% in healthy adults and 1.8% in HIV-infected patients, while the rate of IgG seropositivity (without IgM seropositivity) was 28.7% in healthy adults and 37.8% in HIV-infected patients (p>0.05). Toxoplasma seropositivity was not associated with age, sex, ART status, CD4 cell count or HIV clinical staging. Seventy-four percent of the toxoplasma seropositive HIV-infected patients were asymptomatic and no cases of toxoplasma encephalitis were identified.

Conclusion: Toxoplasmosis is equally prevalent in HIV-infected patients and healthy adults from similar environments in Northern Nigeria. It is imperative to develop public health policies to prevent toxoplasmosis in Nigeria, especially in HIV-infected patients.

Introduction

Toxoplasmosis, a zoonotic parasitic infection caused by *Toxoplasma gondii*, is estimated to have infected about a third of the world's population [1]. In man, *toxoplasma* infection is acquired mainly by ingestion of tissue cysts of the parasite in raw or undercooked meat, by ingestion of parasite oocysts in cat faeces that contaminate soil, vegetables and other food sources, and trans-placentally from infected mothers to their infants [1,2].

After being infected by *toxoplasma gondii***,** the majority of healthy non-pregnant adults develop asymptomatic lifelong latent infection [1,2]. However, in **a** setting of immunosuppression, as seen in human immunodeficiency virus (HIV) infection, there is an increased risk of reactivation of latent infection in various organs, especially in the brain [1,2].

Toxoplasmosis of the brain (also called toxoplasma encephalitis) is one of the most common central nervous system opportunistic infections in HIV-infected individuals and also the most common cause of focal deficits in patients with acquired immunodeficiency syndrome (AIDS) [3-5]. *Toxoplasma*-HIV co-infected patients have a 30% to 40% risk of developing toxoplasma encephalitis, especially those with significant immunosuppression as reflected in a CD4 cell count below 200cells/ul [4,6].

Routine screening for toxoplasmosis involves detection of IgM and IgG antibodies in the patient's serum [1]. In the antibody response to *toxoplasma* infection, IgM antibodies are detected within a few days to one week of infection and disappear generally after three to five months [1,2]. The IgG antibodies are detected within one to two weeks of infection, reaching a peak after four months, then declining to lower levels and remaining positive for the remainder of the individual's life [1,2]. A negative IgM antibody test essentially excludes acute infection while a

positive IgG test with a negative IgM indicates chronic infection [1,2].

Serological studies in man and animals indicate that toxoplasmosis is endemic in most parts of Nigeria [7,8], with seroprevalence rates in humans ranging from 22% to 78% in the general population [7-10]. Few studies from Nigeria evaluating the seroprevalence of toxoplasmosis in HIV-positive and negative non-pregnant adults have been performed [11,12]. However, many aspects of the seroepidemiology of toxoplasmosis the Nigerian population remain undefined. Existing studies of *toxoplasma* infection in HIV-positive and -negative individuals from Nigeria did not evaluate: (i) the prevalence of recent *toxoplasma* infection measured by IgM antibody serostatus; (ii) the relationship between the *toxoplasma* antibody profile and HIV-related variables such as HIV staging, HAART status and CD4 cell counts; and (iii) the clinical characteristics of the *toxoplasma* seropositive HIV-infected patients.

In this study, we sought to cover the highlighted gaps by determining the prevalence and determinants of IgM and IgG antibody response to *toxoplasma* infection in HIV-positive and -negative healthy adults. Results of this study may assist clinicians and policy makers in Nigeria in initiating, planning, and implementing interventions that will ultimately prevent and control toxoplasmosis in Nigeria.

Methodology

In 2008, a cross-sectional study was undertaken in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. The ABUTH is a 700-bed tertiary hospital located in Kaduna State, Northern Nigeria. Approval for the study was obtained from the ABUTH research ethics committee.

After obtaining written consent, a total of 219 non-pregnant adults, consisting of 111 HIV-1 infected adults and 108 HIV-negative healthy adults, were recruited for the study over a six-month period. The HIV-1 patients were recruited consecutively as they presented to the HIV treatment clinic or were admitted into the medical wards of the hospital. The HIV-negative individuals were healthy volunteers resident in similar environments as patients recruited by convenience sampling.

Demographic and clinical data including, age, gender, clinical diagnosis, highly active antiretroviral therapy (HAART) status, as well as WHO HIV clinical stages [13] and CD4 cell counts of study participants were documented.

Laboratory methods

Five millilitres of whole blood samples were withdrawn from each participant by venipuncture. In HIV patients, two millilitres of withdrawn blood was immediately used for CD4 cell count measurement by flow cytometry (Partec, Munster, Germany) according to the manufacturer's instructions. This test kit contains mouse CD4-phycoeryrthrin conjugated monoclonal antibody which recognizes the human CD4 antigen. Residual blood samples from all study participants were spun at 5,000g and the serum samples obtained were used for HIV antibody testing (Capillus, Cambridge Biotech, Galway, Ireland; Determine, Abbott Laboratories United Kingdom; and Immunocomb II Bispot, Orgenics Ltd, Yavne, Israel) and western blot confirmation (Immunetics Qualicode, Boston, MA, USA).The remaining serum samples were frozen at -20° C until analysed collectively for IgM and IgG *toxoplasma* antibodies.

After thawing, all serum samples were screened collectively for both IgM and IgG antibodies to *toxoplasma* infection using an ELISA based kit (Diagnostic Automation Inc., Calabasas CA, USA) according to the manufacturer's instructions. For the IgG and IgM tests, positive results were defined as a value of ≥ 8 international units (IU)/ml.Seropositivity to IgM antibodies (with or without IgG seropositivity) was indicative of acute or recent *toxoplasma* infection while seropositivity to IgG antibodies (with seronegative IgM antibody) was indicative of chronic or latent *toxoplasma* infection.

Study data was analysed using Statistical Programme for the Social Sciences (SPSS) version 17 (IBM SPSS, Chicago, Ill, USA). Chi square, Student's t test and Mann-Whitney U test were used to determine relationships and differences between variables as appropriate. A p value of less than 0.05 was taken as indicative of statistical significance.

Results

Demographic and clinical data

A total of 219 participants consisting of 111 HIV-1 infected adults and 108 HIV-negative healthy adults were included in the study. The HIV-positive participants were aged 19 to 62 years (median age of 35 years; mean ± SD of 35 years ± 9.1), and 72 (64.9%) of them were females. The HIV-negative adults were aged 19 to 60 years (median age of 34 years; mean ± SD of 34 years ± 9.6), and 62 (57.4%) of them were females. Out **o**f the 111 HIV-infected patients, 61 (55%) were HARRT naive, while 30 (27%) had CD4 cell counts less than 200 cells/ul

(median CD4 cell count of 367cells/ul; mean ± SD is 413cells/ul ± 291).

The type of HAART regimen received by the HIV-positive participants included zidovudine/lamivudine/nevirapine (n = 40), tenofovir/emtricitabine/nevirapine (n = 5), zidovudine/lamivudine/efavirenz (n = 1) and tenofovir/emtricitabine/lopinavir/ritonavir (n = 4). With regard to WHO HIV clinical stages, 20 (18%), 20 (18%), 58 (52.3%) and 13 (11.7%) of the 111 HIV-infected patients were in stages 1, 2, 3 and 4 of the disease, respectively.

Seroprevalence of IgM and IgG antibodies to Toxoplasma *infection*

The distribution of IgM and IgG seropositivity to *Toxoplasma* infection is shown in Table 1. There was no significant difference in the seroprevalence of toxoplasmosis (seropositivity to IgM or IgG antibodies or both) in HIV-positive patients (38.7%, 95% CI of 30-48%) as compared to HIV-negative adults (32.4%, 95% CI of 24-42%, P > 0.05, Chi square). The seroprevalence of IgM antibodies, indicating acute or recent *Toxoplasma* infection, and the seroprevalence of IgG antibodies, indicating chronic or latent *Toxoplasma* infection, also did not significantly differ between HIV-positive patients and HIV-negative adults (P >0.05, table 1).

Seroprevalence of toxoplasmosis in relation to demographic and clinical variables

Table 2 shows that there are no significant differences in the seroprevalence of toxoplasmosis (seropositivity to IgM or IgG antibodies or both) in relation to age group, sex, HIV clinical stage, CD4 cell count ranges, or HARRT status in both HIV-positive and negative study participants.

Clinical characteristics of study participants in relation to seropositivity to IgM and IgG antibodies to Toxoplasma *infection*

All HIV-negative adults and 32 (74.4%) of 43 *Toxoplasma* seropositive HIV-positive patients were asymptomatic. Out of the 11 symptomatic patients, one patient was seropositive for IgM antibodies only (a case of primary liver cell carcinoma) and the remaining 10 patients were seropositive for IgG antibodies only. The clinical diagnoses of these patients include *Plasmodium falciparum* malaria (n = 3), pulmonary tuberculosis (n = 2), chronic diarrhoea (n = 2), culture-negative bronchiopneumonia (n = 1), oral thrush (n = 1), and genital warts (n = 1).

None of the seropositive patients had evidence of *Toxoplasma* encephalitis during the study period. Three of the 68 *Toxoplasma* seronegative HIV-infected patients had features of focal deficits, out of which two were cases of hypertensive-related

Table 1. Distribution of IgM and IgG antibody responses to toxoplasmosis in HIV-positive and -negative adults

Antibodies	HIV status					P value
	HIV-positive (n = 111)		**HIV-negative (n = 108)**			
	n (%)	**95% CI %**	**n (%)**	**95% CI %**		
IgM only Acute/recent infection	2 (1.8)	0.3-6.7	5 (4.6)	1.7-11		P > 0.05
IgG only Chronic (latent) infection	42 (37.8)	29-48	31 (28.7)	21-38		P > 0.05
IgM and IgG	1 (0.9)	0.05-6.0	1 (0.9)	0.05-5.8		P > 0.05
Overall IgM and or IgG	43 (38.7)	30-48	35 (32.4)	24-42		P > 0.05

n = number of study participants
CI = confidence interval

Table 2. Toxoplasma seropositivity in relation to demographic and clinical variables of study participants

	HIV status					
	HIV-positive (n = 43)			HIV-negative (n = 35)		
Variable	**n (%)**	**95% CI %**	**P value**	**n (%)**	**95% CI %**	**P value**
Sex						
Male	12 (30.8)	18-48	P > 0.05	18 (39.1)	25-55	P > 0.05
Female	31 (43.1)	22-55		17 (27.4)	17-40	
Age group						
19-29	12 (38.7)	22-58	P > 0.05	12 (26.1)	15-40	P > 0.05
30-40	15 (33.3)	20-49		11 (32.4)	18-51	
> 40	16 (45.7)	29-63		12 (42.9)	25-63	
ART status						
HAART naive	19 (31.1)	20-44	P > 0.05			
Receiving HAART	24 (48)	34-62				
HIV staging						
Early HIV	16 (40)	25-57	P > 0.05			
Late HIV	27 (38)	27-50				
CD4 cell count						
CD4 ≤ 200	10 (33.3)	18-53	P > 0.05			
CD4 >200	33 (40.7)	30-52				

n = number of study participants;
ART = antiretroviral therapy
Early HIV = WHO stages 1 and 2; Late HIV = WHO stages 3 and 4
CI = confidence interval

ischaemic stroke confirmed by clinical history and brain computer tomography, and one was a case of glioblastoma multiforme confirmed by a brain biopsy.

Discussion

Toxoplasmosis is an environmental disease as transmission of the infection has been shown to be promoted by poor environmental sanitation, overcrowding, eating habits, poverty, and poor hygiene, among other factors [10,14,15]. In this study, the seroprevalence of toxoplasmosis in Zaria, Northern Nigeria, was comparable in HIV-positive patients and HIV-negative healthy adults, possibly indicating equivalent exposure to the *Toxoplasma* parasite, since both study groups were selected from similar environments. Our results are supported by other studies from outside Nigeria, including Malaysia [16], Thailand [17], Spain [18], Czechoslovakia [19] and Northern Iran [20], where seroprevalence of toxoplasmosis was found not to be significantly different between HIV-positive and negative adults. However, contrasting findings of significantly higher seroprevalence of toxoplasmosis in HIV-positive patients as compared to healthy HIV-negative adults were reported in Jos, North-Central Nigeria [12] and Lagos, South-West Nigeria [11], as well as in Mali [21], Burkina Faso [22], Uganda [23], and India [24]. Differences in study design, especially in selection of HIV-negative controls, may account for the reported disparities in the seroprevalence of toxoplasmosis in relation to HIV-status.

The IgM antibody response to *Toxoplasma* infection is short-lived and it is frequently suppressed to undetectable levels in the setting of severe immunosuppression [1,2]. In agreement, our study revealed lower rates of IgM seropositivity compared to IgG seropositivity in both study groups, and lower rates of IgM seropositivity in HIV-infected patients as compared to HIV-negative controls. Similar findings of lower rates of IgM seropositivity compared to IgG seropositivity in HIV-positive patients have also been reported by other studies from India [24], Mexico [25] and South Africa [26]. These low rates of detection of IgM antibodies in HIV-positive patients lends support to the view that the screening for this antibody in the routine diagnosis of toxoplasmosis in non-pregnant HIV-infected patients may be of limited value [1,2].

In resource-poor settings, any association between *Toxoplasma* seropositivity and HIV-related clinical variables such as CD4 cell count, ART status and HIV clinical stage may be helpful in classifying patients who may benefit from *Toxoplasma* screening or from prophylaxis against toxoplasmosis. In our study, *Toxoplasma* seropositivity was not related to all these clinical variables, suggesting that in our region screening for toxoplasmosis cannot be recommended based on highlighted HIV-related clinical variables. In agreement with our results, studies from Mexico [25] and Malaysia [16] have shown no correlation between CD4 cell count and *Toxoplasma* seropositivity while another in Nairobi, Kenya [27], reported no correlation between HIV clinical staging and *Toxoplasma* seropositivity. In contrast, Belanger *et al*. [28] in France revealed that HIV patients with CD4 cell counts less than 200cells/μl were more likely to be *Toxoplasma* seropositive than those with counts greater than 200cells/μl.

About 95% of cases of acute and chronic toxoplasmosis present asymptomatically [1]. In agreement, the majority of our *Toxoplasma* seropositive patients were asymptomatic. Although there was no reported case of *Toxoplasma* encephalitis during the six-month study period, the majority of our *Toxoplasma* seropositive patients are at a risk of developing *Toxoplasma* encephalitis, especially if prophylaxis is not offered or when ART failure develops. A case report from South-South, Nigeria [29] and a retrospective case series from South-West Nigeria [30] have confirmed that *Toxoplasma* encephalitis is not uncommon in the HIV-infected population from Nigeria. We are currently undertaking a prospective review to ascertain the actual incidence of *Toxoplasma* encephalitis in our region.

Conclusion

Toxoplasmosis is equally prevalent in HIV-positive and -negative non-pregnant adults from Zaria, North West Nigeria. Latent asymptomatic infections predominate and in HIV-infected patients, *Toxoplasma* infection was not related to CD4 cell counts, HIV clinical stage or ART status. We recommend routine toxoplasmosis preventive education among HIV-infected patients in our region as well as routine screening of all HIV infected patients for IgG anti-*Toxoplasma* antibodies to identify patients at risk of *Toxoplasma* encephalitis.

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