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Toxoplasma gondii IgG antibodies in HIV/AIDS patients attending hospitals in Makurdi metropolis, Benue state, Nigeria

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ABSTRACT

Background: Toxoplasmosis is caused by *Toxoplasma gondii*, a parasite that gradually evolved to be the most opportunistic parasite that complicates the course of HIV/AIDS in developing countries. **Aim:** This study was undertaken to investigate the presence of *Toxoplasma gondii* IgG antibodies in HIV-infected patients attending hospitals in Makurdi metropolis, Benue State, Nigeria. **Materials and Methods:** The Enzyme-linked immunosorbent assay (ELISA) technique was used to determine the presence of Toxo-IgG antibodies in blood samples collected from the HIV/AIDS patients and their CD_4 counts were estimated using flow cytometry. Questionnaires were also administered to obtain information on their socio-demographic status. **Results:** Thirty-nine, 39 (10.8%) were screened positive for Toxo-IgG antibodies out of the 360 HIV/AIDS patients enrolled. Males (10.3%) and females (11.2%) had similar seroprevalence of Toxo-IgG with no significant difference ($\chi^2_=$ 0.001, $p>0.05$). The presence of *Toxoplasma* IgG antibodies was found to be highest in the ≥ 54 years age group. A significant difference was observed in the seroprevalence of *Toxoplasma* IgG among age groups (χ^2=11.56, $p<0.05$). Females with CD_4 T-cell count ≤ 200 cells/mm^3 recorded higher seroprevalence (73.7%) of Toxoplasma IgG. There was no significant difference in the seroprevalence of Toxoplasma IgG in relation to CD_4 T-cell counts ($\chi^2_=$ 2.3, $p > 0.05$). **Conclusion:** Further investigations are still needed to clarify the exact relationship of the parasite infection, its effects on the HIV-infected and uninfected individuals and the detection of their major means of transmission.

Key words: *Toxoplasma*, IgG, antibodies, HIV/AIDS, Hospitals, Nigeria

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan that causes toxoplasmosis and infects both humans and animals.[1] *T. gondii* is a cosmopolitan parasite that is transmitted through: oral ingestion of food or water contaminated with oocysts from cat faeces, oral ingestion of tissue cysts contained in raw or undercooked meat, transplacental

transmission of tachyzoites from the infected mother to her foetus, tachyzoites and tissue cysts via solid organ transplantation.[1] In addition, it has been postulated that the consumption of infected rats may also increase the risk of direct transmission from rats to humans.[1]

Usually, *T. gondii* infection is asymptomatic, however in immunosuppression, the parasites may become widely disseminated and lead to severe toxoplasmosis and encephalitis. An increasing number of parasites was reported to be associated with HIV/AIDS infection. These include: *Pneumocystic carinii* that causes life-threatening pneumonia;[2] *Cryptosporidium*, *Microsporidia, canthamoeba sp, Blastocystis hominis* and *Leishmania sp* that cause severe enteritis, ulceration of the skin and menigoencephalitis.[3,4] Various serological studies around the world reported that *Toxoplasma gondii* infection in HIV infected patients vary from geographical location to the other with prevalences between 8.0% - 97.0%.[5, 6] Over the past two decades and with the present scourge of the human immune deficiency (HIV), *T. gondii* gradually evolved to be the most common opportunistic parasite that complicates the course of AIDS in developing countries. [7-15]

In Nigeria only few studies have dealt with the determination of *Toxoplasma* IgG antibodies in HIV/AIDS patients. However, it has been found that toxoplasmic encephalitis has become one of the most frequent and common cause of focal lesions of the brain complicating the course of AIDS.[16, 17]

There is a paucity of knowledge about *Toxoplasma gondii* infection among HIV-positive patients in Makurdi, Benue State, which is known as one of the leading States with high prevalence of HIV/AIDS patients in Nigeria. However, the indigenous people are found to be eating rats which could be a risk factor that might increase the level of infection in the populace. Thus, this study investigated the seroprevalence of Toxo-IgG antibodies among HIV/AIDS patients attending three hospitals in Makurdi metropolis.

MATERIALS AND METHODS

Study area

The study was done in Makurdi metropolis, Benue state, Nigeria. Makurdi is located at longitude $6^0$28'E and latitude $7^0$14'N of the Guinea savanna zone in central Nigeria. The area experiences the wet season from April to October and dry season from November to March. The area is traversed by the River Benue and divided into the North and South Banks. Residents are primarily civil servants, paramilitary, soldiers, traders, fisherman, farmers and craftsmen.

Study design and sample collection

The study was approved by the Hospitals Institutional Review Board (permission grant No PW/EC/007/004/2010) and was cross sectional in nature. Enrolled patients gave individual consent and were properly educated on the significance of the study. They were also assured of confidentiality.

Blood samples originally meant for CD_4 T-cell counts of HIV/AIDS patients on ART were collected by venipuncture into vacutainouse plane test tubes and allowed to clot. From the 3ml of blood sample given, sera were separated by centrifugation and then frozen at 2 - 8°C prior assayed. Frozen sera were thawed at room temperature and unsuitable ones (based on turbidity and haemolysis) were discarded. Suitable sera were tested in duplicate for anti-Toxoplasma IgG antibody with the use of Enzyme Linked Immunosorbent Assay (ELISA) kit (Micro well Toxo [IgG] EIA Syntron Bioresearch, Inc, CA, USA).

With the manufacturer assay procedures followed strictly, the mean value of Toxo- G Index for each specimen were calculated by dividing the mean absorbance value of each sample by the cut off calibrator mean value. A sample was then considered positive for anti-Toxoplasma IgG antibody whenever a Toxo G Index value is equal or greater than 1.0, and considered negative whenever a Toxo G Index value is equal or less than 0.90.

Assay procedures

In preparation for the assay, the following materials and reagents were used: Micro titre plate reader with optical density (OD) of 450 nm**,** Micro titer pipettes (5 - 200µl)**,** Pipette tips**,** Deionized and Distilled water**,** Well strips coated with inactivated *T. gondii* antigen, Sample diluents**,** Prediluted positive and Negative control**,** Prediluted calibrator**,** Horse Radish Peroxidase (HRP) –anti-human IgG conjugate**,** Washing buffer**,** Tetra- Methyl Benzedine (TMB) Substrate solution, Stopping solution. The reagents were brought to room temperature and mixed well. Also the washing buffer was diluted and mixed well with deionized water.

- A 1:50 dilution of the test samples were made by adding 5µl of the test samples to 250µl of sample diluent in separate tubes.
- And with a multichannel pipette, 100µl of Positive and Negative control, calibrator, and each diluted sample from the tubes were transferred to the Wells.
- The Wells were then covered and incubated at 37°C for 30 minutes, after which the liquid from the Wells were shaken vigorously out of the Well and then washed 5times with diluted washing buffer.
- 100µl of HRP conjugate solution were then added to each Well, incubate for 30 minutes at 37°C and washed thoroughly with diluted washing buffer.
- 100µl of the TMB substrate solution was then added to each Well and incubated for 10 minutes at room temperature.
- After which 100ul of stopping solution was added to each Well and gently shaken.
- Finally, a 450nm set wavelength Micro plate Reader was used to measure the Optical density (OD) of each Well and reported as follows:

To determine the positivity or negativity of each serum specimen of patient, the specimen optical density (OD) ratio was determined by dividing specimen optical density by the optical density of the calibrator, and interpreted as shown below.

$$Specimen\ OD\ ratio = \frac{Specimen\ OD}{Calibrator\ OD}$$

Specimens	OD Ratio Range	Inferences
Positive	Greater or equal to1.0	Current/previous infection
Negative	Less or equal to 0.90	No detectable antibody
Equivocal	0.91 – 0.99	Specimen to be retested

Statistical analysis

A computer program SPSS (Statistical Package for Social Sciences, version 17.0) was used to analyse collected data. Chi-square test was used to compare *Toxoplasma gondii* infection among sexes, age group, the three hospitals and CD_4 ranges of the patients. The 5% level of significance was used.

RESULTS

Table 1 shows the distribution of Toxo-IgG in relation to sex and location of hospitals in Makurdi metropolis, Benue State, Nigeria. An overall seroprevalence of 39(10.8%) was observed out of the 360 HIV/ AIDS patients screened. Males and females were equally infected with 10.9% (16/147) and 10.8% (23/213) respectively as evidenced by the seropositivity of *Toxoplasma* IgG antibodies ($\chi^2_{=}$ 0.001, *p>0.05*). In relation to the hospitals location, General Hospital (G.H) North Bank recorded higher seroprevalence,15 (12.5%) than Bishop Murray Medical Center (BMMC), High level, 13 (10.8%) and Federal Medical Centre (FMC), Wadata, 11 (9.2%). There was no significant difference in the seroprevalence of Toxo-IgG in the HIV-infected patients in the three locations (χ^2=0.0690; *p > 0.05*).

Fig 1 shows the age-group distribution of Toxo-IgG in HIV-infected patients in Makurdi metropolis, Benue State, Nigeria. Seroprevalence of Toxo-IgG varied between 0.0%-30.3% with the age group ≤ 13 years having the lowest seroprevalence (0.0%) and the age group ≥ 54 years having the highest, 30.3%. There was a significant difference in the distribution of Toxo-IgG between the different age groups ($\chi^2_{=}$ 11.6, *p<0.05*).

Table 2 shows the distribution of Toxo-IgG in relation to CD_4 T-cell counts in HIV-infected patients in Makurdi metropolis, Benue State, Nigeria. Females with CD_4 T-cell counts < 200 cells/mm^3 recorded the highest seroprevalence of Toxo-IgG with 73.7% (14/19), while both sexes recorded similar seroprevalences for CD_4 T-cell counts > 200 cells/mm^3. There was no significant difference in the distribution of Toxo-IgG in relation to CD_4 T-cell counts in the HIV-infected patients ($\chi^2_{=}$ 2.3, *p > 0.05*).

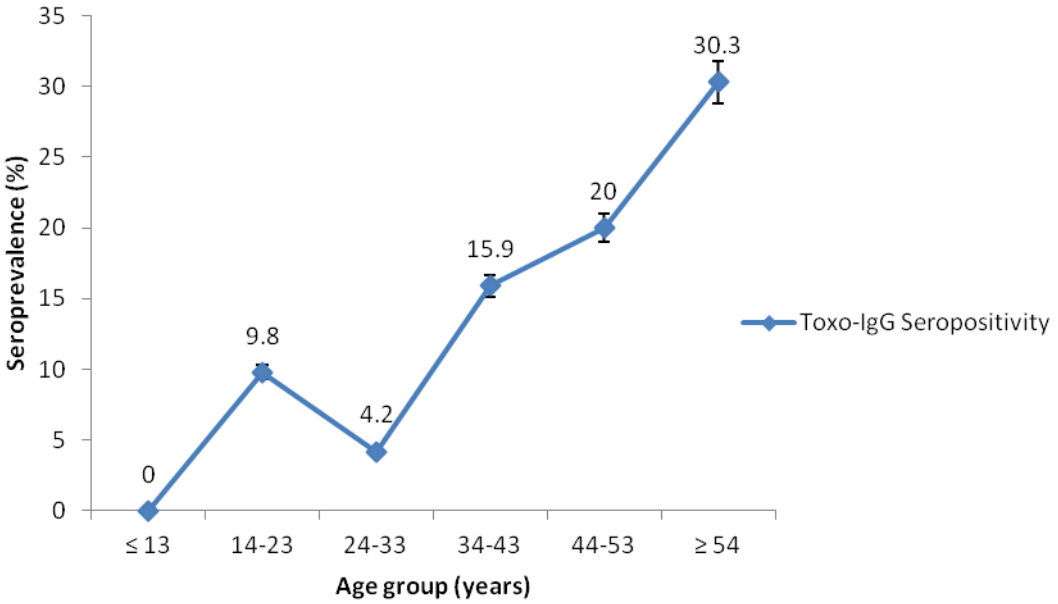

Fig 1 :Age-distribution of Toxo-IgG seropositivity in HIV-infected patients in Makurdi metropolis, Benue state, Nigeria ($\chi^2_{=11.6,}$ $p<0.05$)

Table 1: Distribution of Toxo-IgG in HIV-infected patients in relation to sex and location of hospitals in Makurdi metropolis, Benue State, Nigeria

	Males		Females		Total	
Hospitals'location	**No. Exam**	**No.pos (%)**	**No. Exam**	**No. pos(%)**	**No.Exam**	**No.pos(%)**
BMMC (High Hevel)	46	5(10.9)	74	8(10.8)	120	13(10.8)
GH (North-Bank)	52	6(11.5)	68	9(13.2)	120	15(12.5)
FMC (Wadata)	49	4(8.1)	71	7(9.8)	120	11(9.2)
Total	**147**	**15(10.2)**	**213**	**24(11.3)**	**360**	**39(10.8)**

Keys: No. Exam = Number examined
No. pos = Number positive

Table 2: Distribution of Toxo-IgG in relation to CD_4 T-cell counts in HIV-infected patients in Makurdi metropolis, Benue State, Nigeria.

CD_4 counts (cells/mm^3)	Male (%)	Female (%)	Total
≤ 200	5(26.3)	14(73.7)	19
201-400	8(50.0)	8(50.0)	16
401-600	2(50.0)	2(50.0)	4

$\chi^2 = 2.3$, $p>0.05$

DISCUSSION

The seroprevalence of Toxoplasma IgG antibodies in HIV-infected patients in Makurdi metropolis was found to be 10.8%. This seroprevalence was lower than reports observed by Alanyade *et al*.[19] and Osunkalu *et al*.[20] who reported seroprevalence of 26.1% and 58% among HIV-individual in Sokoto State and HIV-infected patients attending Lagos State University Teaching Hospital respectively. This lower seroprevalence could be the result of environmental and climatic factors which could not favour the transmission of *Taxoplasma gondii*. The study was conducted during the dry season (January-March) which is known as the hottest period where temperature could reach the highest peak (38º-40º C). Several studies reported variations in seroprevalence of toxoplasmosis either within the same geographical location or within urban and rural settings. These variations could be due to the exposure of individuals through their various activities (occupation) or their various living conditions (poor and rich settlements) with unhygienic and hygienic conditions. When compared to studies reported from other countries, the seroprevalence of Toxo-IgG in infected HIV-infected patients in Makurdi seems to be low. In Cameroon a neighbouring country to Nigeria, a seroprevalence of 42.1% was reported among HIV-patients attending the University Teaching Hospital Yaoundé.[21] Nissapatorn *et al*.[14] reported seroprevalence of 51.2% among HIV-infected patients in hospital, Kuala Lumpur, Malaysia. In Iran, Morazh *et al*.[22] reported a seroprevalence of 49.7% among HIV-positive patients.

With regards to the hospitals' location, General Hospital (GH), North-Bank recorded the highest seroprevalence of Toxo-IgG (12.5%) among the HIV-infected patients. Though this result is not significant, but this could clearly demonstrate the living conditions of the inhabitants in this area. The area is found to be insalubrious with no hygiene, presence of refuse dumps littering the environment, unclean gutters, uncovered wells and bushes surrounding the houses. These could make the rats to be striving and living together with inhabitants. In addition, this area is found to be accommodating several fast food joints where undercooked meat is found thus favouring transmission. The area is also inhabited by the indigenous people (Tiv) who are fond of eating rats this can favour direct transmission from the rats to human. This has been postulated by Olusi *et al*.[1] among rats eating people in Benue state, Nigeria.

In relation to sex, females were found to be similarly infected (11.3%) with males (10.2%). This shows that males and females were equally exposed to *Toxoplasma gondii* in the area. The results obtained in this study collaborates previous studies that reported similar trends among males and females among HIV-infected patients in Jos, Nigeria[23] and at Seoul National University Children's Hospital, South Korea.[24] In contrast, this result disagrees with the study of Nissapatorn *et al*.[14] who reported a significant higher positive rate in males (48.0%) than females (9.3%) in HIV-infected patients at Hospital Kualar Lumpur, Malaysia.

The seroprevalence of *T. gondii* infection among HIV-infected patients in Makurdi metropolis was observed to increase with age, with the lowest seroprevalence (0.0%) recorded in the age-group ≤13 years old and the highest (30.3%) in the age-group ≥ 54 years. This shows that the latter age group appeared to be more predisposed to *T. gondii* infection than the

younger ones. Patients in these age-groups are prone to be infected through the consumption of undercooked meat (beef or pork) prepared in various favourite cultural food that are usually consumed. Besides this, the traditional beliefs that meats are only meant for adults may be responsible for the increased seroprevalence in the older age group. The higher seroprevalence of *T. gondii* infection in this age group may also be due to lower immunity level than in the younger patients. Similar observations were also observed among HIV-infected patients in Iran with the age group ≤ 10 years old having the lowest seroprevalence (0.0%) and the age group ≥ 51 years having the highest (75.0%).[22] This result agrees with the findings of Falusi *et al*.[25] who observed higher seroprevalence (35.3%) in the age group ≥ 50 years. This finding disagrees with reports of Banerje *et al*.[26] who reported seroprevalence of 5.2% among the ≤ 13 years old. The reason for the rise in quantitative titres with age may not be clearly defined among the age groups; the reason for these variations in the seroprevalence may be due to the increased chance of an individual coming in contact with one of the routes of transmission.

This study shows no significant association between CD_4 T–cell count and Toxo-IgG although more females with CD_4 count ≤ 200 cells/mm^3 recorded the highest seroprevalence (73.7%). In clinical practice CD_4 T-cell count need to be considered as a prognostic or risk factor to monitor the progression and possible development of toxoplasmosis in HIV–infected patients.

Further investigations are still needed in Benue State to clarify the exact relationships of the parasite infections, its effects on the HIV-infected and uninfected individuals and the detection of their major means of transmission in the area and Nigeria at large.

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Conflict of Interest: None declared