ORIGINAL ARTICLE

Seroprevalence of *Toxoplasma gondii* among HIV Patients in the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria

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ABSTRACT

Background: Toxoplasmosis among the human immunodeficiency virus (HIV) positive individuals with low CD4+cell count can be severe leading to the reactivation of dormant bradyzoites which can lead to morbidity and/or mortality. A reservoir for T. gondii, cat is common around our study area both as pets and stray animals.
Study Objective: Is to determine the seroprevalence of T. gondii infection in this community and also to investigate the association between T. gondii infection and CD4 count other risk factors.
Methods: Blood specimens were collected from a total of 282 adult HIV positive patients attending a tertiary health facility clinic by systematic sampling and the IgG and IgM levels were detected using the enzyme-linked immunosorbent assay (ELISA) technique. Similarly blood was also collected from 60 healthy blood donors as control group.
Results: Out of a total of 282 samples analyzed, 189 were positive for IgG (67%) and 93 were negative. All 282 samples were negative for IgM antibody. A total of 280 had their CD4+cell count above 200cells/microlitre of blood and only 2 recorded less than 200. Nineteen (31.7%) of the 60 blood donors in the control group were positive for IgG and all negative for IgM.
Conclusion: The high seroprevalence rate of IgG toxoplasma antibody among HIV positive patients as reported in this study suggests that there should be a high index of suspicion of the recrudescence of toxoplasmosis among HIV and AIDS patients in our environment and possibly elsewhere.

KEYWORDS: Seroprevalence, *Toxoplasma gondii*, HIV positive patients.

Introduction

Toxoplasma gondii is a protozoan parasite that can infect virtually all warm-blooded animals including humans. It causes the disease toxoplasmosis which is a zoonosis. It is estimated that about 20% to 90% of the world adult population must have had contact with the parasite.[1-4]

The members of the cat family felidae are the only known definitive hosts for its sexual stages and thus are the main reservoir of infection.[5-7]

The parasite was first described in 1908 when found in the blood, liver, and spleen of a North African rodent, Ctenodactylus gondii. It was then named Toxoplasma (arc-form) gondii (after the rodent) and it is one of the most prevalent chronic infections that man has to contend with. [8]

Toxoplasmosis in the immunocompetent individuals is mostly asymptomatic; whereas in the immunocompromised individuals such as HIV infected and transplant recipients on immune-suppressive therapy can lead to severe infections.[9]

Human infection may be acquired in several

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ways; ingestion of undercooked or raw meat containing T. gondii cysts, Ingestion of the oocyst from faecally contaminated hands, food, or water, organ transplantation or blood transfusion, transplacental transmission and accidental inoculation of tachyzoites (trophozoites).

The two main routes of transmission of Toxoplasma gondii to humans are oral and congenital because T. gondii organisms are rarely detected in humans with toxoplasmosis, serologic examination is used to indicate the presence of the infection by detecting Toxoplasma specific antibodies. [10,11]

It is a known fact that reactivation of chronic infection is the most common cause of toxoplasmosis in patients with AIDS, malignancies, or organ/tissue transplants; initial assessment of these patients should routinely include an assay for T. gondii IgG antibodies. IgG antibody testing should be ideally performed as soon as it is established that the patient is immunocompromised or about to be immunosuppressed. Those with positive result are at high risk of reactivation of the infection.[12-15] It is appropriate to determine the seroprevalence of Toxoplasma gondii antibodies in a given community. Toxoplasmosis is a rare disease among HIV positive individuals CD4 cell count above 200 cells per microlitre of blood and it is most common among those with CD4+ cell count below 50 cells per microlitre of blood. Jacques et al [16] reported a strong association of Toxoplasma gondii seroprevalence with HIV serostatus while Nahlen et al [17] associated very low CD4+ cell count with central nervous system toxoplasmosis HIV infection/AIDS. We were obliged to conduct this study considering the prevalence and scourge of HIV/AIDS in developing nations of the world especially in sub-Sahara Africa and the fact that at very low CD4 level, dormant bradyzoites of T. gondii can be reactivated leading to morbidity and mortality. This type of study has not been previously done in our community to the best of our knowledge.

Materials And Methods

Study Centre

This study was conducted at the HIV outpatient clinic, University of Benin Teaching Hospital, Benin City, Nigeria. It is one of the clinics implementing the Federal Government of Nigeria anti-retroviral (ARV) access programme. HIV-care, treatment and support is given at this centre.

Sample Size (N)

282 consenting individuals attending the clinic were recruited into the study together with 60 healthy blood donors as control group. The sample size was estimated using the standard cross-sectional sample size formula where P is the expected prevalence rate, Z the value of the reference normal distribution for the desired confidence level (1.96) for 95% confidence level. D is the highest acceptable error in the estimate.

$$N = \frac{Z_2 [P(1-P)]}{D^2}$$

P = 83% (0.83); Z = 1.96; D = 5% (0.05)

Inclusion Criteria and Exclusion Criteria

Adult HIV positive males and females including pregnant women that were systematically sampled, and enrolled for this study; however all children were excluded. Also, all HIV negative adults were exclusive with the exception of the control group which comprises of HIV negative blood donors.

Ethical Clearance

The UBTH ethical committee approved the conduct of this study.

Study Design

A cross-sectional study of 282 participants was conducted from September, 2015 to March, 2016 in Anti-retroviral (ARV) clinic of University of Benin Teaching Hospital. Data was collected from individuals that were accessing anti-retroviral treatment from the ARV clinic.

Data Collection
A structured questionnaire was administered to each consenting participant to assess the socio-demographic characteristics, variable risk factors for Toxoplasma gondii infection among the study participants. CD4-Cell count of each individual was extracted from the patient's clinic folder in order to evaluate its association with laboratory findings.

Laboratory Procedure
2.5mls blood sample was collected at the HIV treatment clinic of the University of Benin Teaching Hospital, Benin City. This was followed by laboratory analysis of the samples; the sera were tested for IgG and IgM antibodies to Toxoplasma gondii using Toxoplasma ELISA- based test kits (Rapid Labs Ltd, Colchester Essex, Co 7, 8SD, UK); Lots: 1503028 and 1501002 for IgG and IgM respectively.

Data Management
Data was entered and analysed using SPSS 20 software. Statistical test result was considered significant whenever P value was <0.05.

Results
A total of 282 blood specimens of HIV positive patients were examined for Toxoplasma gondii specific IgM and IgG. IgM was negative for all the samples tested. However, 125(44.3%) males and 91(55.7%) females were positive for IgG antibody respectively.

The seroprevalence for males is 73% and 62% for females. This gives a combined seroprevalence of 67% (Table 1). There was no significant difference between the prevalence rate of the males and females (p? 0.05). One hundred and seventy six patients aged 36 and above had prevalence of 72-77%; while those between 18 and 35 had prevalence between 51 and 57%. This constitutes a significant difference in the prevalence between these age groups (? 0.05) (Table 1). Nineteen (31.7%) of the 60 blood donors in the control group were positive for IgG and all negative for IgM. CD4+⩾ 200 cell/microlitre of blood is recorded for 125 males and 153 females. Only 2 females with CD4+cell count less than 200 (197 & 188) (Table 2). The socio-demographic characteristics shows that most of the seropositives are in the lower and middle strata thus: Traders 79(42%), agricultural workers 57(30%) and others including the unemployed 40(21%), they collectively, amount to 90% of the total seropositive participants; while the government workers most of whom constitute the high socioeconomic strata recorded 13(7%) (p>0.05). There is no significant difference in the level of education of those that are seropositive: Primary, 36(19%), secondary 115(61%), and tertiary 20(38%) (p>0.05).A total of 188(99.5%) of them responded positively to ownership of cat at home or/and stray-cat visiting home. (Table 3).

TABLE 1: Seroprevalence of T. gondii in the UBTH

Variable	No. Examined	No. Positive	Prevalence %	P value
Sex				
Male	125(44.3%)	91	73	>0.05
Female	157(55.7%)	98	62	
Age group				
18 –25	43	22	51	>0.05
26– 35	63	36	57	
36- 45	94	72	77	
⩾46	82	59	72	

TABLE 2: CD4 cell count

Range	Sex M	F	p-value
CD4 ⩾ 200	125	155	< 0.05
CD4 < 200	0	2	

Table 3: Socio-Demographic Characteristics of Seropositive Patients for *T.Gondii*

Variable	No. (Prevalence)	p-value
Occupation		
Government workers (including civil servants)	13(7%)	< 0.05
Traders/merchants	79(42%)	< 0.05
Agricultural workers	57(30%)	
Others/unemployed	40(21%)	
Level of education		
Primary	36(19%)	> 0.05
Secondary	115(61%)	
Tertiary	20(38%)	
Risk factors for Toxoplasmosis		
Ownership of Cat at home or	Yes…. 188(99.5%)	
Stray-cat visiting home	No…. 1(0.5%)	Nil
Eating of pork/raw meat	Yes…. 2(1.09%)	Nil
	No…. 187(98.91%)	
Eating of improperly washed	Yes… 1(0.5%)	
Vegetables/fruits	No … 188(99.5)	Nil

Discussion

Toxoplasma gondii infection is a known zoonostic disease that is common all over the world and its transmission is facilitated by poor personal and environmental sanitation, poverty, overcrowding and the habit of eating raw or undercooked meat.[18,19] *Toxoplasma gondii* specific IgM and IgG antibody levels were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA); which is one of the standard methods for the detection of anti-*Toxoplasma gondii* antibodies[20]. Both IgG and IgM levels were measured because this will provide information as to whether the infection is acute/active or chronic. This study determined the seroprevalence of *Toxoplasma gondii* antibodies in HIV positive patients in the Niger-Delta region of Nigeria with healthy blood donors as control group. And the overall prevalence was found to be 67% and 34% respectively. There is a significant difference ($p<0.05$) between the prevalence rates in both groups, This is in agreement with the findings of Akanmu et al,[21] in Lagos who recorded 54% and 37.5% for HIV positive patients and immunocompetent

control group respectively. Uneke et al [22] in Jos got 38.8% and 20.8% prevalence rates for the HIV positives and control group respectively similar to the findings of Ogoina D et al[23] who also recorded low seroprevalence in Zaria, Northern Nigeria among HIV positive persons and negative control group, thus 37.8 and 32.4% respectively. The low prevalence in Jos and Zaria may be due to discrepancy in the population of infected animal reservoirs such as cat in the Northern and Southern regions of Nigeria. Serological studies conducted in human show that the seroprevalence rates of latent *Toxoplasma gondii* infections are variable and also studies conducted within the same country can exhibit variable outcome depending on geographical and cultural variation. Very low seroprevalence rates are recorded in Far East countries (about 1%) and very high rates in some parts of Europe and South America (>90%).[24] There is no significant difference in the prevalence in respect of age group (P>0.05). This is in variance with the findings of Papoz et al [25] and Okwuzu et al [26], both found highest prevalence in lower age groups probably due to increase exposure to soil that must have been contaminated with *T. gondii* oocysts. Most of the patients we studied belong to the low and middle-level socioeconomic strata and therefore their personal/environmental hygiene is expected to be low and this may favour transmission of the parasite. Contraction of the infection can be as easy as ingesting oocysts after handling contaminated soil with cat litters or consumption of contaminated water and food. This could in part account for the high prevalence rate recorded in this study. Virtually all the participants in this study had past or present history of owning a cat or stray cat visiting their homes or places of work especially at night. Similarly Nneke et al [22] recorded high level of seropositivity among people associated with ownership of cat/home visiting by stray cats. This means that their immediate environment is likely to be contaminated with the parasite. All the patients (males and females) had CD4+cell count $\geqslant$ **200** and 2 females had <200. This may justify why 0% seroprevalence of IgM was recorded. Earlier studies asserted that the toxoplasmosis is a rare disease among HIV positive persons with CD4 count >200 and most common among those with count <50.[15]

Conclusion

This study shows a high rate of seroprevalence of IgG Toxoplasma antibody among HIV positive patients; which means that healthcare givers to these patients should always bear in mind that toxoplasmosis is a likely opportunistic infection among patients who develop AIDS. This study describes the prevalence of *Toxoplasma gondii* antibody in HIV patients in a tertiary healthcare facility which may not be representative of the entire Niger-Delta region though our study centre the UBTH covers a vast catchment area with over 600- bed capacity. We recommend for a multicentre study which is expected to be more inclusive.

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