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TOXOPLASMA GONDII INFECTION IN HIV/AIDS: PREVALENCE AND RISK FACTORS

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§ correspondence: E-mail ogoamakajane@yahoo.com**ABSTRACT****BACKGROUND:** Toxoplasmosis is an infection caused by the protozoan *Toxoplasma gondii*. It is common in severely immunocompromised persons.**OBJECTIVE:** To determine the seroprevalence of *T. gondii* infection and the risk factors associated with the infection and to investigate the association between *T. gondii* infection and CD4 cell count.**METHODS:** Sera collected from 242 HIV positive HAART-naïve patients were tested for *T. gondii* specific immunoglobulin G antibodies. Information was obtained using a structured questionnaire. Baseline CD4 cell counts were obtained from patients' case files. Data was managed using SPSS version 20 software and Microsoft Excel worksheet.**RESULTS:** One hundred and sixty eight (69.4%) subjects were females while 74(30.6%) were males. One hundred (41.3%) of study participants were *Toxoplasma* IgG antibody positive. Thirty two(32) HIV positive pregnant women were among this group studied with 12(37.5%) being Toxo IgG antibody positive. *Toxoplasma* seropositivity was higher in females (42.8%) than in males (39.2%), $P= 0.60$. CD4 cell count level of < 200 was negatively associated with *Toxoplasma* seropositivity than CD4 count ≥ 200 by logistic regression (OR= 0.6; 95% CI 0.3- 1.0). Living in proximity with cat was positively associated with *T. gondii* infection ($P= 0.01$).**CONCLUSION:** *T. gondii* infection is common in pregnant women indicating greater probability of congenital transmission of *T. gondii*. This could form a basis for recommending intensifying health education and prophylactic treatment for all HIV positive pregnant women. Measures should be taken to prevent stray cats from entering homes.**Keywords:** *Toxoplasma gondii* , IgG, Seroprevalence, HIV positive, CD4 cells.**INFECTION A TOXOPLASMA GONDII CHEZ LES PERSONNES VIVANT AVEC LE VIH / SIDA : PREVALENCE ET FACTEURS DE RISQUE**

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§ Adresse Mail d'auteur correspondant: E-mail ogoamakajane@yahoo.com**RÉSUMÉ****Contexte:** La toxoplasmose est une infection causée par le protozoaire *Toxoplasma gondii* . Il est fréquent chez les personnes gravement immunodéprimées. **Objectif:** Déterminer la séroprévalence de l'infection à *T. gondii* et les facteurs de risque associés à l'infection et à étudier l'association entre l'infection par *T. gondii* et la numération des CD4. **Méthodes:** Les sérums prélevés à partir de 242 sujets séropositifs au VIH et naïfs au HAART ont été testés pour des anticorps immunoglobulines G spécifiques de *T. gondii*. L'information a été obtenue à l'aide d'un questionnaire structuré. Le nombre de cellules CD4 de base ont été obtenus à partir des dossiers des patients. Les données ont été analysées avec les logiciels SPSS version 20 et Microsoft Excel.**Résultats :** Cent soixante-huit (69,4%) sujets étaient des femmes alors que 74 (30,6 %) étaient des hommes. Cent (41,3 %) des participants à l'étude étaient positifs à des anticorps IgG de *Toxoplasma*. Trente deux (32) femmes enceintes séropositives ont été parmi ce groupe étudié avec 12 (37,5%) étant anticorps IgG Toxo positive. La séropositivité de *Toxoplasma* était plus élevé chez

les femmes (42,8%) que chez les hommes (39,2%), $P = 0,60$. Le niveau de $CD4 < 200$ a été négativement associé à la séropositivité de *Toxoplasma* que le comptage de $CD4 \geq 200$ par régression logistique (OR = 0,6, IC 95% 0,3-1,0). Vivre à proximité du chat était positivement associée à l'infection par *T. gondii* ($P=0,01$).

Conclusion: L'infection à *T. gondii* est commune chez les femmes enceintes indiquant une plus grande probabilité de la transmission congénitale de *T. gondii*. Cela pourrait constituer une base pour recommander l'intensification de l'éducation sanitaire et le traitement prophylactique pour toutes les femmes enceintes séropositives. Des mesures devraient être prises pour empêcher les chats errants de pénétrer dans les habitations.

Mots-clés: *Toxoplasma gondii* , IgG , séroprévalence , VIH positif , les cellules CD4

INTRODUCTION

Toxoplasmosis is a disease caused by the protozoan *Toxoplasma gondii*, which affects humans in the brain causing *Toxoplasma* encephalitis and other organs like eyes and lungs. *T. gondii* encephalitis could be via acute infection or reactivation of latent infection among immune suppressed persons including those with acquired immuno-deficiency syndrome, those with immunosuppressive cancer and transplant recipients on immunosuppressive drugs. Toxoplasmosis occurs in 3-10% patients in USA and up to 50% of patients in Europe and Africa (1). In patients with AIDS, CNS involvement is the most common manifestation, ranging from nonspecific, generalized symptoms to focal findings such as headache, altered levels of consciousness, motor impairment, and seizures. Seizures was reported as an early manifestation of cerebral toxoplasmosis (2). Various studies have revealed different incidences; 12-25% toxoplasmosis in HIV infected persons (2, 3). Adults with normal immune function are usually asymptomatic or might have symptoms such as fever, malaise and lymphadenopathy that resolve spontaneously. The disease is rare among HIV positive people with T- cell counts of above 200 and most common among HIV positive people with T-cell count below 50(4). Similar study by Jacques et al., 2006 reported a strong association of *T. gondii* seroprevalence with HIV serostatus. Nahlen et al., (1992) (6) also associated very low CD4+ cell counts with CNS toxoplasmosis in HIV/AIDS.

Congenital Toxoplasmosis is the most serious form of human infection. Intra-uterine infection with *T. gondii* due to active parasitaemia during pregnancy can cause severe and fatal damage to a foetus. Fetal infection with *T. gondii* may result in stillbirth or abortion. Infection could also lead to fetal brain damage or mental retardation, blindness, and epilepsy in infancy or much later in life. CNS involvement with *Toxoplasma gondii* is uncommon in HIV infected children. Rate of transplacental transmission has been reported to be 55% for untreated mothers and 25% for treated mothers (7). Approximately 10-20% of pregnant women infected with *T. gondii* show clinical signs. Congenital infection

is most severe if acquired in the first or, in some cases, second trimester. Infection during the second or third trimesters tends to be asymptomatic. Seventy-five percent of infants born with congenital toxoplasmosis infection are asymptomatic. Eight percent show severe CNS impairment, which might not manifest for several years.

Serologic prevalence data indicate toxoplasmosis as one of the most common human infections throughout the world, more common in warm climates. Prevalence of 25.3% was reported in pregnant women in Burkina Faso (5), 22.5% in USA with 15% seroprevalence found among women of child bearing age (8). Seroprevalence in Nigeria was reported as 78% among pregnant women in Ibadan (9) and 83% in the population of South Delta in Nigeria (10).

Common sources of this infections are: eating of raw or undercooked meat containing *T. gondii* tissue cysts or eating food that has been cross- contaminated; ingesting of oocysts from soil through gardening, handling and eating unwashed vegetables or changing a cat litter box, through placenta (congenital infection), and through sexual contact as suggested by Jacques et al., 2006 in the study of HIV, HBV, HCV and *T. gondii* co-infection. Studies revealed presence of *T. gondii* antibodies in the animal populations and suggested that toxoplasmosis is a widely spread zoonotic infection(11, 12, 13).

Evidence has shown *T. gondii* infection to cause complication in children born of HIV infected pregnant woman, with latent infection reactivated when immunity is suppressed. This poses a serious health threat especially in our country Nigeria with high prevalence of HIV/AIDS. Hence the need for the study in HIV/AIDS patients in our clinic and this could form a basis for primary prophylaxis.

Objectives

1. Determine the prevalence of toxoplasmosis in HIV patients including pregnant women.
2. Identify risk factors to toxoplasmosis in HIV patients.

- To find association between toxoplasmosis and T cell counts.

MATERIALS AND METHODS

Study centre

This study was conducted at the HIV counseling and testing (HCT) unit of the outpatient clinic, Nigerian Institute of Medical Research, Yaba, Lagos. It is one of the clinics implementing the federal government of Nigeria anti-retroviral (ARV) access programme with support from Harvard School of public health. HIV care, treatment and support is given at the center.

Sample size

242 consenting individuals attending the clinic for the first time was recruited in the study. This 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
All new HIV positive males and females including pregnant women were included in this study, however non- consenting HIV positive patients and infants of < 18 months were excluded.

Ethical clearance

Nigerian Institute of Medical Research ethical review board approved the study.

Study

A cross-sectional study of 242 participants was conducted from April, 2009 to April, 2012 in Anti-

retroviral (ARV) clinic, N.I.M.R Yaba, Lagos. Data was collected from individuals on first visit to assess anti-retroviral treatment from ARV clinic, NIMR.

Data

A structured questionnaire was administered to each consenting participant to assess the socio-demographic characteristics, variable risk factors to *Toxoplasma gondii* infection by the individual. T- Cell count of each individual was extracted from the patient's clinic folder in order to evaluate its association with laboratory findings.

Laboratory

2mls blood sample was collected at the HCT centre of NIMR. This was followed by laboratory analysis of the samples; the serum was tested for IgG antibody to *Toxoplasma gondii* using Toxoplasma ELISA- based test kit (TECO Diagnostics, USA).

Data

Data was entered and analysed using SPSS 20 software.

RESULTS

Of the 242 HIV positive patients tested between 2009 and 2012, 100 (41.5%) were positive for T. *gondii* IgG antibody. Toxoplasmosis prevalence by sex, age group, ethnic group and level of education is shown in table 1. *Toxoplasma gondii* IgG seroprevalence decreased across the group with age group < 21-30 being the highest (58.9%). The prevalence was also higher in females (42.8%) than in males (39.2%). Among 32 HIV-positive pregnant women, 12(37.5%) were positive for T. *gondii* IgG antibody. Only the factor, 'living in proximity with cats' was significantly associated with T. *gondii* seropositivity (Table 2). However, Mean CD4 count was 273 cells/ μ l; Min- 6 cells/ μ l, Max- 1407cells/ μ l. CD4 level of <200 was a greater predictor of seropositivity than \geq 200 by logistic regression analysis (OR = 0.6; 95% C.I 0.3 - 1.0).

TABLE 1: PREVALENCE OF TOXOPLASMOSIS BY DEMOGRAPHY

Demographic characteristics	Prevalence N (%)	P- value
Sex		0.603
Male	29 (39.2)	
Female	71 (42.8)	
Ethnic group		0.885
Hausa		
Igbo	4(50)	
Yoruba	48(43.2)	
Others	23(39)	
	24(39.3)	

TABLE 2: RISK FACTORS OF TOXOPLASMOSIS

Risk factors	Frequency (%)	P- value
Sex partners		0.103
Faithfulness	74 (40.2)	
> 1 partner	17 (45.0)	
None	8 (36.4)	
Cat ownership		0.208
Yes	4 (66.7)	
No	96 (41.0)	
Living in proximity with cat		0.013
Yes	29 (56.9)	
No	71 (37.6)	
Eat raw pork/meat		0.290
Yes	10 (52.6)	
No	88 (40.2)	
Drink unpasteurised milk		0.768
Yes	1 (33.3)	
No	99 (41.8)	
Indulge in		0.881
Yes	6 (40)	

DISCUSSION

From the result, the prevalence was higher in females than in males; with majority (89%) of the females

Demographic characteristics	Prevalence N (%)	P- value
Level of Education		0.475
None		
Primary	9(64.3)	
Secondary	21(42.5)	
Tertiary	47(41.6)	
	23(38.3)	

gardening	No	94 (42)	
Soil related occupation	Yes	6 (30)	0.258
	No	93 (43.1)	
Eat unwashed vegetables and fruits	Yes	12 (40)	0.843
	No	88 (41.9)	
Eat unwashed veg. and fruits outside home	Yes	38 (41.3)	0.991
	No	60 (41.1)	
Wash kitchen knife after use	Yes	91 (41.4)	0.917
	No	6 (40)	
Wash hand before eating	Yes	99 (41.9)	0.396
	No	0 (0)	
Source of drinking water	Pipe borne water	5 (23.8)	0.228
	Borehole	84 (43.5)	
	Pure water	11 (50)	
Boil, filter or use water guard	Yes	42 (38.2)	0.314
	No	58 (44.6)	

from this study being of childbearing age. This suggests that pregnant women should take appropriate precautions to protect themselves against

this infection. Such precautions include cooking meat, especially lamb and pork, until it is well done; thorough washing of cutting boards used to prepare meat; wearing gloves when gardening; rigorous hand washing after handling raw meat or working in the soil; and avoiding contact with cat feces. From this study, 37.5% of the pregnant women were positive to *T. gondii* IgG antibody. We were not able to confirm recent infection in the women using anti- *T. gondii* IgM for possible congenital transmission. However, IgG avidity testing is the preferred method to confirm recent infection (14), because IgM antibodies can persist for months after initial infection in some individuals. It has been shown that 43 cases of toxoplasmosis acquired during pregnancy would be expected to result in 11–21 cases of congenital toxoplasmosis, assuming a 25–50% probability of transmission to the fetus in the uterus (15).

The *T.gondii* IgG seroprevalence (41.5%) from this study was similar to that (38.8%) obtained in Jos, Nigeria (16) among HIV positive individuals. Prevalence of 37.5% observed in pregnant women was higher than what was obtained in pregnant women (25.3%) in Burkina Faso (5) and 22.5% in USA (8). However, our result is lower than what was obtained (78%) among pregnant women in Ibadan, Nigeria (9) and 83% in the population of South Delta in Nigeria (10). It could be an improvement in the control strategies against *Toxoplasma gondii* infection that brought about the reduction in prevalence in the high risk group. Highest prevalence obtained in the lower age group as was highlighted by Papoz et al., (1986) probably reflected soil exposure to *T. gondii* oocysts. Living in proximity with cat was positively associated with *T. gondii* seropositivity. Others studies have associated eating of rodents, having soil related

occupation (16), cat ownership etc. with seropositivity. This is understandable because in our environment, most people do not own cats (table 2) but we see cats gaining access to people's homes especially in the night where they look for leftover food leaving their droppings to contaminate the surfaces. It is encouraging to note the hygienic practice of the study participants and these goes to show that infection in this group would have been from eating of improperly cooked meat/ meat products, vegetables and fruits, though it was not significant, especially outside home. Earlier studies have acclaimed that the disease is rare among HIV positive people with T- cell counts of above 200 and is most common among HIV positive people with T-cell count below 50(4). T- cell count group < 200 from this study is associated to *T. gondii* seropositivity than above or equal to 200.

In conclusion, *T. gondii* infection is common in pregnant women indicating greater probability of congenital transmission of *T. gondii*. This could form a basis for recommending intensifying health education and prophylactic treatment for all HIV positive pregnant women. Screening of newborns over a specified time period could elucidate the true burden of congenital toxoplasmosis. Measures should also be taken to prevent stray cats from entering homes.

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REFERENCES

1. Ravindra K. G. HIV infection and seizures. *Postgrad Med J.*1999; 75: 387-390.
2. Holtzman D. M, Kaku D. A, So Y. T. New -onset seizure associated with human immunodeficiency virus infection: causation and clinical features in 100 cases. *American Journal of Medicine*, 1989; 87: 173-177.
3. Dore A. J, Law M.G, Brew B.J. Prospective analysis of seizures occurring in human immunodeficiency virus type-1 infection. *Journal of Neurology AIDS*, 1996; 1: 59-69.

4. Sellbrink H.I, Fuhrer-Burrow R, Raedler A, Albrecht H, Fenske S. Risk factors for severe Disease due to *Toxoplasma gondii* in HIV- positive patients. *European Journal of Epidemiology*, 1993.9 (6): 633-637.
5. Jacques S, Savadogo, Denise I, Nadambega M, Esposito, M, Salvatore P, Pietra V, Salvatore M. *Toxoplasma gondii*, HCV and HBV seroprevalence and co- infection among HIV positive and negative pregnant women in Burkina Faso. *Journal of Medical Virology*, 2006; 78(6): 730-733.
6. Nahlen B, Sorvillo F, Farizo K. Seroprevalence of toxoplasmosis among HIV infected adults in Los Angeles, California. *International conference on AIDS*. 1992, July 19-24; 8:111 (abstract no- PUB 7375).

7. Joseph S. Toxoplasmosis. *E medicine- infectious diseases*, 2002. 1.
8. Jeffrey L. J, Deanna K, Marianna W, Geraldine M, Thomas N, James B. M. *Toxoplasma gondii* infection in the united states: Seroprevalence and risk factors. *Am J Epidemiol*, 2001. 154(4): 357-365.
9. Onadeko M. O, Joymson D. H, Payne R. A. The prevalence of *Toxoplasma* infection among pregnant women in Ibadan, Nigeria. *Journal of Tropical Medicine and Hygiene*; 1992. 95: 143- 145.
10. Dubey J. P, Beattie C. P. Toxoplasmosis in man (*Homo sapiens*). In: *Toxoplasmosis of animals and man*. Boca Raton, FL: CRC press, Inc. 1988.: 41-60.
11. Sharif M, Gholami S. H, Ziael, Daryani B, Lankfarce S, Ziapour S. P, Rafiei A and Vahedi M Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats slaughtered for food in Mazandaran province, Iran, during 2005. *The Veterinary Journal* 2007; 174(2): 422- 424.
12. Van der Puije, K. M, Bosompen, K. M, Canacoo E. A, Wasting J. M and Akanmori B. D. The prevalence of anti- *Toxoplasma gondii* antibodies in Ghanaian sheep and goats. *Acta Tropica* 2000; 76(1): 21-26.
13. Ivana K, Olgica D. D, Sofijakatic R, Aleksandra N. Cross- sectional survey on *Toxoplasma gondii* infection in cattle, sheep and Pig in Serbia: Seroprevalence and risk factors. *Veterinary Parasitology* 2006; 135: 121- 131.
14. Sickinger E, Gay-Andrieu F, Jonas G, Schultess J, Stieler M, Smith D, Hausmann M, Stricker R, Stricker R, Dhein J, Braun H B. Performance characteristics of the new ARCHITECT Toxo IgG and Toxo IgG avidity assays. *Diagnostic Microbiology Infectious Disease* 2008;62:235-244.
15. Abu-Madi Marawan A, Behnke Jerzy M., and Dabritz Haydee A. *Toxoplasma gondii* Seropositivity and Co-Infection with TORCH Pathogens in High-Risk Patients from Qatar. *American Society of Tropical Medicine and Hygiene* 2010; 82(4): 626-633.
16. Uneke C.J, Duhlinska D.D, Njoku M.O, Ngwu B.A. Seroprevalence of acquired toxoplasmosis in HIV-infected and apparently healthy individuals in Jos, Nigeria. *Parassitologia* 2005; 47(2): 233-236.
17. Papoz L, Simondon F, Saurin W. A simple model relevant to toxoplasmosis applied to epidemiologic results in France 1986. *Amer J of Epidemiol*; 123: 154-61.