



Full Length Research Paper

Seroprevalence and risk factors of *Toxoplasma gondii* infection (toxoplasmosis) among hiv seropositive pregnant women in a tertiary healthcare centre, Kano, Northern Nigeria

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Abstract

Toxoplasmosis caused by an obligate intracellular Protozoan, *Toxoplasma gondii* has emerged as an important cause of opportunistic infection in HIV/AIDS resulting in severe morbidity and significant mortality. HIV infected pregnant women are prone to infection by *T. gondii* due to immune suppression and may lead to severe complications and congenital abnormalities. However, data on this subject matter are scarce in this environment. The study was aimed to determine the seroprevalence of *T. gondii* infection among HIV infected pregnant women and associated risk factors in Kano, northern Nigeria. A total of 273 HIV seropositive pregnant women attending Aminu Kano Teaching Hospital (AKTH), Kano were screened for *T. gondii* antibodies (IgG/IgM) by serology including determination of CD4 cell level in the patients by standard procedures. Socio-demographic information and risk factors variables of Toxoplasmosis including patient history were obtained via a questionnaire. Data were analyzed statistically. The overall (chronic and acute infections) seroprevalence of *T. gondii* infection in HIV pregnant women was 34.1%; IgG, 30.8% versus IgM, 3.3%. The age of patients ranged between 18-39 years (mean age, 29.13± 5.01). The highest seroprevalence (39.8%; IgG, 36.7% versus IgM, 3%) was found among the patients aged 31-40 years. Majority of the patients with chronic *T. gondii* infection (IgG, 72.4%) and acute infection (IgM, 10.3%) were found in those with CD4 cells count/μl of <200, and 200-349 CD4 cell count/μl respectively. Data analysis showed that the subjects with regular contacts with cats and their litters including consumption of poorly prepared meat products were significantly associated with *T. gondii* infection (P < 0.05). The high (34.1%) seroprevalence of *T. gondii* infection and chronicity (IgG, 30.8%) in HIV infected pregnant women is concerning due to associated maternal and fetal complications. There is need for routine universal and antenatal screening for *T. gondii* in HIV seropositive pregnant women that will aid early detection and treatment to limit the spread of the disease and associated morbidity and mortality.

Keywords: Toxoplasmosis, seroprevalence, immunosuppression, pregnant women, morbidity, mortality.

INTRODUCTION

Toxoplasmosis caused by an obligate intracellular Protozoan, *Toxoplasma gondii* is an emerging infectious parasitic disease with increasing public health significance (Tenter *et al.*, 2000; Caruthers, 2002). In the last two decades, *T. gondii* has evolved as an important

cause of opportunistic infections in HIV/AIDS patients resulting in severe morbidity and significant mortality (Luft *et al.*, 1992; James *et al.*, 2009).

Macleod (1988) and Mims, *et al.* (1998) reported that *T. gondii* has high potential to cause serious complications

in HIV pregnant women, who are also predisposed to variety of other infections during gestational period due to immune suppression. Infection by *T. gondii* during pregnancy may lead to severe congenital abnormalities including premature delivery or still birth (Onadeko *et al.*, 1996).

T. gondii infection is widespread among human population. Globally, the incidence of Toxoplasmosis varies among different population group (including HIV seropositive pregnant women) and ranges between 16-80% in different localities (Tenter *et al.*, 2000). It accounts for 38% of opportunistic central nervous system (CNS) infection, and 50-60% of intra-cerebral mass lesion in HIV/AIDS patients (Arora *et al.*, 2009; Kistiah *et al.*, 2011). In Nigeria, seroprevalence of Toxoplasmosis ranging from 16-30% have been reported among the general population (Mitchel *et al.*, 1994; Onadeko *et al.*, 1996). However, data on the burden of *T. gondii* infection among HIV infected pregnant women in this environment are scanty hence the need for the present study. The study was aimed to determine the seroprevalence of *T. gondii* and associated risk factors among HIV seropositive pregnant women in Kano, northern Nigeria. It is hoped that the outcome will further sensitize the populace on the need to adopt effective control measures that will checkmate the transmission of *T. gondii* infection among especially the HIV infected pregnant women to limit associated complications.

MATERIALS AND METHODS

Study Area

The study was carried out at Professor Sadiq Wali HIV treatment centre of Aminu Kano Teaching Hospital, Kano (AKTH). AKTH is a tertiary health care institution located in the large cosmopolitan city of Kano, northwest Nigeria. The hospital serves as a referral centre to the population of Kano state and neighboring states of Jigawa, Kastina, and parts of Kaduna.

Professor Wali centre is a large, well-equipped HIV/AIDS treatment clinic with over 18,000 registered patients and daily turn-over of 200 patients. It is supported by the Institute of Human Virology of Nigeria (IHVN); the President's Emergency Programme for AIDS Relief (PEPFAR, USA) project. The centre has both clinical and laboratory facilities for the screening/diagnosis and follow up of HIV infected patients. It also supports the prevention of mother to child transmission of HIV/AIDS programme (PMTCT).

Study Design

This was a cross sectional, prospective study involving 273 HIV seropositive pregnant women attending AKTH,

Kano, Nigeria between the month of January to December 2014, who gave consent for the study. The minimum sample size used in this study was obtained using the formula and procedure as described by Lwanga *et al.* (1991).

Data Collection

In each case, a questionnaire consisting of personal data and relevant risk factor variables of *T. gondii* infection was administered to record patient's socio-demographic information including name, age, occupation and educational qualifications, the kind of pets kept and methods of care; the type of food regularly prepared or procured and mode of preparation and consumption.

Assays

HIV Serology

The HIV status of the pregnant women was determined based on standard guidelines with DETERMINE HIV 1/2 (Abbott Comp. Ltd., Tokyo, Japan). All reactive samples were retested with STAT-PAK (Caldon Biotech, Inc., Carlsbad, CA, USA) for confirmation and reliability of results.

CD4 Cell Count

Ten millilitres (mls) of blood sample was collected from each patient by venipuncture and immediately emptied into a pre-labeled EDTA bottle. Three mls of the blood sample was withdrawn and immediately used for CD4 cell measurement by flow cytometry technique (Partec, flow, Germany). The manufacturer's instructions were followed methodically, and results were interpreted accordingly.

Toxoplasma (*T. gondii*) Serology

The remaining blood sample (7 mls) was centrifuged at 3000 rpm for 10 minutes and serum obtained. All sera collected were transferred into pre-labeled cryovials and stored at -20 °C until analyzed. IgG and IgM were detected using the IgG/IgM ELISA kit (Diagnostic automation Incorp. Carlsbad, CA, USA). Results were interpreted according to the manufacturer's instructions.

Data Analysis

Data were analyzed with Epi info (version 6.04, CDC, Atlanta, GA). The prevalence of *T. gondii* infection was expressed in simple proportion or percentages for the study group. Comparison of the group regarding the prevalence of infection, association between parasite

Table 1: Seroprevalence (IgM and IgG) of *Toxoplasma gondii* among HIV infected pregnant women in Kano.

Age range (Yr.)	No examined	No (%) positive for:		Total no (%) positive
		IgM	IgG	
11-20	10	1(10)	4(40)	5 (50)
21-30	165	5(3)	44(26.7)	49 (29.6)
31-40	98	3(3)	36(36.7)	39 (39.8)
Total	273	9(3.3)	84(30.8)	93 (34.1)

Table 2: Seroprevalence of *T. gondii* in relation to CD4 cell counts of the patients

CD4 Cells/ μ l	IgG		IgM	
	Positive	Negative	Positive	Negative
	n (%)	n (%)	n (%)	n (%)
<200	71(72.4)	27(27.6)	1(1.0)	97(99.0)
200- 349	4(10.3)	35(89.7)	4(10.3)	35(89.7)
\geq 350	9(6.6)	127(93.4)	4(2.9)	132(97.1)
Total	84(30.8)	189(69.2)	9(3.3)	264(96.7)

infection and CD4 cell count and risk factors were analyzed using Chi-square test or two-tailed Fisher's exact tests for categorical variables and Student's t test for continuous variables. A p-value of < 0.05 was considered statistically significant. The approval to conduct the study was sought and granted by the Ethical committee of the hospital (AKTH).

RESULTS

For this present study, a total of 273 HIV seropositive pregnant women aged 18-39 years; mean age, 29.13 \pm 5.01, were screened for *T. gondii* antibodies. The overall seroprevalence was 34.1% with IgG and IgM antibodies constituting 30.8% and 3.3% respectively (Table 1). The highest seroprevalence of 39.8% (IgG, 36.7%; IgM, 3.1%) was found among the patients aged 31-40 years. Majority of the patients with chronic *T. gondii* infection (IgG, 72.4%) and acute infection (IgM, 10.3%) were found in those with CD4 cell count/ μ l of < 200, and between 200-349 CD4 count/ μ l respectively. The seroprevalence of Toxoplasma infection was significantly high (72.4%) in those with CD4 cell/ μ l of < 200 for IgG antibodies. Whereas in the analysis of IgM antibodies against *T. gondii*, the highest prevalence was found in patients whose CD4 cell count/ μ l falls between 200 to less than 350. Significant association ($p < 0.05$) was found between *T. gondii* infection and CD4 cell counts for both IgG and IgM (Table 2).

Analysis of risk factors of Toxoplasmosis shows that HIV seropositive pregnant women with regular contact

with cats were significantly associated ($p = 0.002$) with chronic *T. gondii* infection (IgG; $p = 0.04$). However, on the basis of individual antibody seroprevalence, the highest prevalence of IgG (40%) and IgM (10%) was detected among the patients in the age bracket 11-20 years (Table 1). Those who consistently handled cat litters were found to have significantly associated with chronic *T. gondii* infection (IgG; $p=0.02$).

DISCUSSION

Toxoplasma gondii is a coccidian Protozoan found worldwide (Arora *et al.*, 2009). Infection with *T. gondii* (Toxoplasmosis) is believed to be widespread (incidence rate may be $\geq 80\%$ in some localities) though with relative low mortality in immune-competent hosts (Bata *et al.*, 2009). It is however, associated with severe morbidity, aggravated congenital defects in infected developing foetus, and increased mortality in immune compromised individuals such as HIV/AIDS (Luft *et al.*, 1992; Onadeko *et al.*, 1996). Emmanuel *et al.* (2011) reported that there are variations in the rate of occurrence of Toxoplasmosis in different localities and regions, and may be influenced by some risk factors.

In the present study, the overall seroprevalence (due to detection of IgG and IgM antibodies) of *T. gondii* among HIV seropositive pregnant women was 34.1 percent (Table 1). There was a preponderance (prevalence, 30.8%) of chronic or latent form of Toxoplasmosis (due to presence of IgG antibodies) among infected pregnant women screening during the

Table 3: Seroprevalence of *T. gondii* in relation to the risk factors

Risk Factors	Total No. of Subjects	Total No. Positive (%)	IgG(%)	IgM(%)
Contact with cats				
Yes	103	66 (64.1)	62 (60.2)	4(3.9)
No	170	27(15)	22(12.1)	5(2.9)
Total	273	93(87.8)	84(81.2)	9(6.6)
Handling of Cat Litters				
Yes	87	62(71.2)	59(67.8)	3(3.4)
No	186	31(16.6)	25(13.4)	6(3.2)
Total	273	93(87.8)	84(81.2)	9(6.6)
Poorly cooked Meat				
Yes	148	85(57.4)	78(52.7)	7(4.7)
No	125	896.4)	6(4.8)	2(1.6)
Total	273	93(79.10)	84(72.3)	9(5.3)
Source of Water				
Pipe	171	61(35.7)	52(30.4)	9(5.3)
Well	99	32(32.2)	32(32.3)	0(0.0)
Pond	3	0(0.0)	0(0.0)	0(0/0)
Total	273	93(68)	84(62.7)	9(5.3)

Table 4: Seroprevalence of *T. gondii* according to socio-demographic characteristics of the HIV seropositive pregnant women

Characteristic	Total No. of Subjects	Total No. Positive (%)	IgG(%)	IgM(%)
Age (Yr.)				
11-20	10	5(50.0)	4(40.0)	1(10.0)
21-30	165	49(29.7)	44(26.7)	5(3.0)
31-40	98	39(39.8)	36(36.7)	3(3.1)
Total	273	93(34.06)	84(30.7)	9(3.30)

study (Table 1). This result is comparable with the prevalence of 29.1% and 31.5% previously reported by Bata and his colleagues (2009), and Emmanuel *et al.* (2011) respectively. However, it is lower than the figures ranging between 32.2% and 35.1% of IgG prevalence published elsewhere by various authors (Zumla *et al.*, 1991; Abu-Madi *et al.*, 2010; Deji-Agboola *et al.*, 2011). On the other hand, the seroprevalence of acute Toxoplasmosis (eliciting the presence of IgM antibodies) constitute 3.3 percent. This result falls within the range of 2.6-5.2% reported by other investigators elsewhere outside Nigeria (Nijem, 2009; Abu-Madi *et al.*, 2010; Cheesbrough, 2010).

The seemingly difference in the prevalence rates being reported by various researchers was probably due to the variation in the level of exposure to the risk factors of the disease by sampled population; their geographical location (as it relates to weather condition viz atmospheric temperature and humidity which affect the survival of *T. gondii*) (Kistiah *et al.*, 2011), methodology (Pelloux *et al.*, 1998) and sample size (Kistiah *et al.*, 2011).

A significant association ($P < 0.05$) was observed between *T. gondii* infection and CD4 cell count in both

chronic and acute cases (Table 2). The incidence of Toxoplasmosis was high in those patients with CD4 cell count of less than 200 cell/ μ l. Similar observations had been reported elsewhere in Nigeria (Deji-Agboola, 2011) and outside the country (Koskiniemi *et al.* 1989). This is not surprising as HIV infection is well known to contribute to the depletion of T-lymphocytes (CD4 cells) sub-population and reduce immunity which predisposes the victims to various forms of opportunistic infections including *T. gondii* (Nijem, 2009).

Several studies (Tenter *et al.*, 2000; Bata *et al.*, 2009; Deji- Agboola *et al.*, 2011) have shown human Toxoplasmosis to be dependent upon life style including keeping of pets and their management, sources of drinking water, nature and quality of animal products consumed and level of literacy among the populace. In previous reports (Nijem, 2009; Abu-Madi *et al.*, 2010), regular contact with domestic animals such as cats and their litters were linked with *T. gondii* infection. We also observed similar scenario in the present study. For instance, majority of our subjects (66, 64.2 %) had regular contact with domestic cats, while 60.2% (62 subjects) were regularly exposed to cat litters in their residents. Most positive cases for chronic or latent

Toxoplasmosis were also recorded among these subjects (Table 3). Similarly acute cases of *T. gondii* infection were observed among exposed subjects but at reduced level of infection.

In the same manner, identical results were recorded among the patients who, at one time or another, consumed poorly prepared meats and/or having dug well as their main source of drinking water in their localities. The findings are in agreement with other reports where Toxoplasmosis has been associated with water borne transmission from especially well water contaminated with *T. gondii* (Bata et al., 2009; Sroka, 2010).

Transmission of *T. gondii* is believed to be predominantly common in rural areas and among those without any form of formal or western education as similarly observed in the present study (Table 4) (Koskiniemi et al., 1989).

The explanation of the previous authors was that, educated individuals were more enlightened and tend to observe greater personal and environmental hygiene than members of a community who never had any formal education and/or are consistently living in the rural areas where the parasite thrives. This may account for higher *T. gondii* infection in this group as was observed in the present study.

CONCLUSION

The high seroprevalence (34.1 %) of *T. gondii* infection among HIV infected pregnant women is concerning due to associated maternal and fetal complications including congenital defects. There is need for routine antenatal screening for *T. gondii* infection in HIV seropositive pregnant women for early detection and chemotherapy to limit associated morbidity and complications.

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REFERENCES

- Abu-Madi MA, Behnke JM, Prabhaker KS, Al- Ibrahim R, Lewis JW (2010). Intestinal Helminthes of Feral Cat Populations from Urban and Suburban District of Qatar. *Vet. Para.* 168: 284-292.
- Alvarado-Esquivel C, Estrada-Martínez S, Liesenfeld O (2011). *Toxoplasma gondii* Infection in Workers Occupationally Exposed to Unwashed Raw Fruits and Vegetables: A Case Control Seroprevalence Study. *Parasite Vect.* 16:224-235.
- Arora DR, Arora B (2009). *Toxoplasma gondii* In: Medical Parasitology 2nd edition, CBS Publishers, New Delhi, p. 83-88.
- Bata SI, Ikwe A, Jalatau UU, Idris L, Randawa AJ (2009). Seroprevalence and Risk Factors for *Toxoplasma gondii* Infection Among Antenatal Women in Zaria, Nigeria. *Res. J. Med. Medic. Sc.* 4(2): 483-488.
- Caruthers VB (2002). Host Cell Invasion by the Opportunistic Pathogen, *Toxoplasma gondii*. *Acta. Trop. J.* 81: 111-122.
- Cheesbrough M (2010). Diagnosis of Toxoplasmosis. District Laboratory Practice in Tropical Countries, Part 1. Second Edition Update. Cambridge University Press. Cambridge P. 300-309.
- Deji-Agboola AM, Busari OS, Osinupebi OA, Amoo OJ (2011). Seroprevalence of *Toxoplasma gondii* Antibodies Among Pregnant Women Attending Antenatal Clinic of Federal Medical Center, Lagos, Nigeria. *Intern. J. Biol. Med. Res.* 2(4): 1135 – 1139.
- Emmanuel CU, Raymond A, Jude O, Hannah E, Lawrence E (2011). Comparative Seroprevalence and Risk Factors of Toxoplasmosis Among Three Subgroups in Nigeria. *J. Natur. Sc. Res.* 3: 8-10.
- James DC, Gail JH, Sheldon LK, William JS, Peter JH (2009). *Toxoplasmosis Causes, Diagnosis and Treatment*, 5th Edition, Elsevier Publisher, Amsterdam. p. 2986-3005.
- Kistiah KBA, Winiecka-Krusnell J, Karstaedt A, Freaun J (2011). Seroprevalence of *Toxoplasma gondii* infection in HIV positive and HIV Negative Subjects in Gauteng, South Africa. *South Afri. J. Epidemiol. Infect.* 26 (4): 225-228.
- Koskiniemi M, Lappalainen M, Hedman K (1989). Toxoplasmosis Needs Evaluation, An Overview and Proposals. *Am. J. Dis. Child.* 143:724-728.
- Luft BJ, Remington JS (1992). Toxoplasmic Encephalitis in AIDS Patients. *Clin. Infect. Dis.* 15: 211-222.
- Lwanga S, Lemeshow S (1991). Sample Size Determination in Health Studies: A Practical Manual. World Health Organization, Geneva
- Macleod CL (1988). *Parasitic Infections in Pregnancy*. C.L. Macleod (Editor), Oxford University Press, Oxford.
- Mims C, Playfair J, Roitt I, Wakelin D, William R (1998). *Obstetric and Perinatal Infection In: Medical Microbiology*, 2nd edition, Mosby International Ltd. London, p. 287-312.
- Mitchell CD, Pizzo PA, Wilfert CM (1994). *Toxoplasmosis In: Paediatric AIDS; the Challenge of HIV Infection in Infants, Children and Adolescents*. 2nd edition. Williams and Wilkins Publishers, Baltimore. p 419-431.
- Nijem K (2009). Seroprevalence and Associated Risk Factors of Toxoplasmosis in Pregnant Women in Hebron District, Palestine. *East.Mediterr Health J.* 15:1279-1284.
- Onadeko MO, Johnson DH, Payne RA, Francis J (1996). The Prevalence of Toxoplasma Antibodies in Pregnant Women and the Occurrence of Stillbirth and Congenital Malformations. *Afr. J. Med. Medic Sc.* 25:331-334.
- Pelloux H, Burn E, Vernet G, Marciat S, Jolivet M (1998). Determination of Anti-*Toxoplasma gondii* Immunoglobulin G Avidity: Adaptation to the VidasSystem. *Diagn. Microbiol. Infect. Dis.* 32: 69-73.
- Sroka JW, Szymanska J, Dutkiewicz J, Zajac V, Zwolinski J (2010). The Occurrence of *Toxoplasma gondii* Infection in People and Animals from Rural Environment of Lublin Region - Estimate of Potential Role of Water as a Source of Infection. *Ann. Agric. Environ. Med.* 17(1):125-132.
- Tenter AM, Heckeroth AR, Weiss LM (2000). *Toxoplasma gondii*: from Animals to Humans. *Intern J. Parasitol.* 30: 1217-1258.
- Zumla A., Savva D, Wheeler RB (1991). Toxoplasma serology in Zambian and Ugandan Patients Infected with the Human Immunodeficiency Virus. *Transact. Roy. Soc. Trop. Med. Hyg.* 85: 227-9.