Seroprevalence of Toxoplasmosis and Immunological Status of Human Immunodeficiency Virus (HIV) Infected Pregnant Women in Kano, Northwestern Nigeria

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Abstract: Human Immunodeficiency Virus (HIV) infected pregnant women are susceptible to a variety of infections including toxoplasmosis with high potential of the parasite to cause severe complications. This study was aimed at determining the seroprevalence of toxoplasmosis among HIV infected pregnant women in Kano-Nigeria. The study was conducted at Aminu Kano Teaching Hospital (AKTH). A cross sectional study was used to recruit 273 HIV seropositive pregnant women for the study. Standard procedures were used to screen these patients for *T. gondii* antibodies (IgG/IgM) including determination of the CD4 cell counts. The seroprevalence was found to be 30.8% for IgG and 3.3% for IgM while the overall prevalence was 34.1%. The prevalence of anti-toxoplasma IgG according to the age of the subjects showed highest prevalence (40%) among the age group 11-20. Those from the rural areas had the highest prevalence of 33.3% and 5.3% both for IgG and IgM compared to the urban dwellers. There is a significant statistical association between the latent infection of *T. gondii* (IgG) and contact with cats (P=0.00). Strong relationship between IgG and poorly cooked meats was further established (P=0.00). Seroprevalence of *toxoplasma gondii* infection is higher (72.4%) in those with CD4 cell count of <200 cells/mls. The results of this study indicated that HIV infected pregnant women could be at higher risks of Sero-conversion from chronic form (IgG) of *T. gondii* infection to the acute form (IgM) and that the detection of IgM antibodies is of great concern because IgM is associated with serious consequences on the fetus.

Keywords: Toxoplasmosis, HIV infected pregnant women, Seroprevalence, *Toxoplasma gondii*

INTRODUCTION

Human Immunodeficiency Virus (HIV) infected pregnant women are susceptible to a variety of infections including toxoplasmosis with high potential of the parasite to cause severe complications (Feleke *et al*., 2019) Trans-placental transmission of toxoplasmosis may be a cause of abortion, stillbirth, and serious congenital anomalies (Kheirandish *et al*., 2019).

Toxoplasmosis is an infection of homoeothermic vertebrates, caused by the obligate intracellular protozoan parasite, *Toxoplasma gondii (T. gondii)*. Its broad host range, high infection rate, worldwide distribution and the ability to maintain a benign coexistence with its host are features of *T. gondii* which allows it to be widely regarded as one of the most successful parasites on earth (Montazeri *et al*., 2020).

During acute toxoplasmosis, symptoms are often influenza -like: swollen lymph nodes, muscle aches and pains that last for a month or more (Feleke *et al*., 2019). Rarely will a human with fully functioning immune system develop severe symptoms following infection except in young children and immune-compromised people, such as those with HIV/AIDS, those taking certain types of chemotherapy, or those who have recently received an organ transplant and pregnant women may develop severe toxoplasmosis (Feleke *et al*., 2019).

In Nigeria, few documented literatures reported the prevalence of *T. gondii* to range from 31.5% to 72.5% in pregnant women (Karshim & Karshima (2020); Emmanuel *et al.* (2013).-Additionally, recent demographic data of groups at risk especially pregnant and HIV infected women are insufficient and in developing countries, *Toxoplasma gondii* screening is not part of the health tests included in the routine prenatal assessment. Thus, there is an urgent need to update the existing literature on the prevalence of *T. gondii* among HIV infected pregnant women in an effort to reduce the risk of complications and congenital transmission.

MATERIALS AND METHODS

Study Area

The study was carried out at Prof. Sadiq Wali HIV Treatment Centre, a specialized antiretroviral treatment clinic located inside Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria (11.9664° N, 8.5487° E). It has a registered number of 18,000 clients with daily turnover of 200. It is supported by the Institute of Human Virology of Nigeria (IHVN) and the President's Emergency Programe for AIDS Relief (PEPFAR) project with grant from United State of America Centre for Disease Control (CDC). The approval to conduct the study was sought and granted by the Ethical committee of the hospital (AKTH).

Study Design

A cross sectional study was used to recruit 273 HIV seropositive pregnant women who gave consent for the study. The minimum sample size was obtained using the formula described by Lwanga and Lemeshow (1991).

Data Collection

A pre-structured questionnaire that covered three sections; the socio-demographic data, the major risk factors and history of complications was used to collect the required information of the patients.

HIV Serology

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

Determination of CD4 Cell Count

The CD4 Cell Count of the blood samples was determined according to the manufacturer's instructions (Partec GmBh, Germany). Twenty µl of CD4 antibody and 20 µl of the venous whole blood were added into a partec test tube and incubated in the dark for 15 minutes at room temperature with gentle mixing every 5 minutes during the incubation. Eight hundred µl of CD4 buffer was added mixed gently and plugged onto the CyFlow SL_3 machine which automatically processed the sample and printed out the CD4 result.

Detection of Toxoplasma (*T. gondii*) using Serology

The presence of *T. gondii* in the blood sample was carried out according to the manufacturer's instructions (Diagnostic Automation Inc, Calabasas CA, USA). Exactly 50ml of the wash buffer was diluted with 1L of water. The test sera, calibrator and control sera were diluted 1:81 (10 µL + 800 µL) for IgM; 1: 21 (10 µL + 200 µL) for IgG in serum diluent plus. One hundred µL of diluted patient sera, calibrator and control were then added to the individual wells, while 100 µL of serum diluent plus was added to the reagent blank well and incubated for 30 minutes at room temperature. An automated washing machine was used to wash the wells five times, and then blotted on paper toweling removing all the liquid from the wells. One hundred µL of conjugate was then added to each well and incubated for 30 minutes again at room temperature. Washing was repeated five times, and then 100 µL of tetra-methyl-benzidine (Chromogen/Substrate) was added to each well and incubated at room temperature for 15 minutes. One hundred µl of stop solution was added to stop the reaction.

Data Analysis

Data generated were analysed 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 infection and CD4 cell count and risk factors were analysed 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.

RESULTS

A total of 273 HIV infected pregnant women were screened for anti-toxoplasma IgG and IgM antibodies. The seroprevalence was found to be 30.8% for IgG and 3.3% for IgM while the overall prevalence was 34.1% (Figure 1).

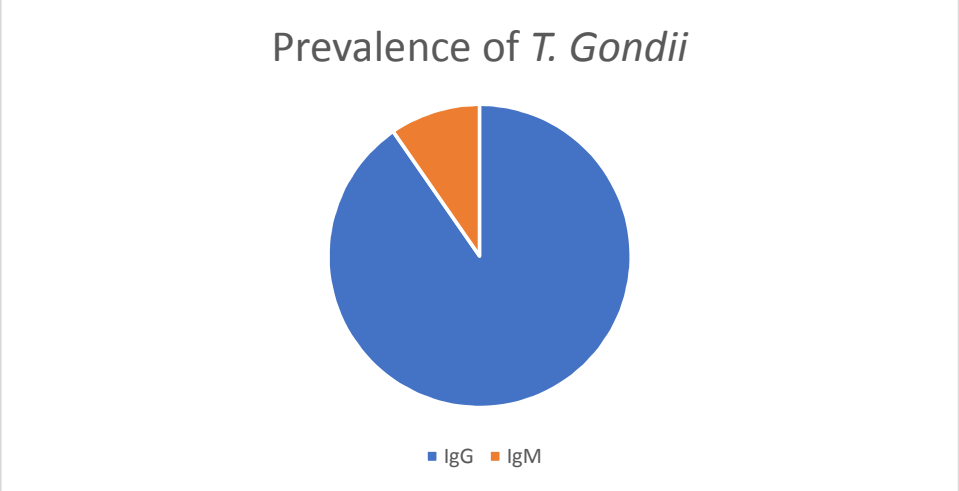

Figure 1: Seroprevalence of Anti- Toxoplasma Antibodies IgG and IgM

The age of the participants ranged between 18-39 years. The mean age of the subjects was 29.13 ± 5.014. More than half of the subjects 165 (60.4%) are in the age group of 21-30 years. The result of the study shows that the highest prevalence of anti-toxoplasma IgG and IgM was recorded among those aged 11-20 years with 40% and 10% prevalence respectively (Table 1). Seventy-eight (28.6%) of the study population had up to secondary school level of education. Qur'anic, primary and tertiary level of education constitute 27.5 %; 17.2% and 26.4%, respectively. The seroprevalence in relation to level of education of the subjects was found to be highest in those with only Qur'anic education both in the case of IgG and IgM (38.7% and 5.3%) (Table 1). Most of the subjects 198 (72.5%) were urban residents while 75 (27.5 %) reside in the rural area. Those from the rural areas had the highest prevalence 33.3% and 5.3% both for IgG and IgM (Table 1).

Those who use well as their source of drinking water had the highest prevalence (32.3%) (Table 2). One hundred and three (37.7 %) of the subjects had contact with cats (Table 2). There is a significant statistical association ($p<0.05$) between the latent infection of *T. gondii* (IgG) and contact with cats. Multiple exposure to cats occurred in 88 (32.2 %) of those who had contact with cats and the exposure had occurred less than a month in 93 (34.1 %) of the subjects. Significant association ($p<0.05$) between multiple exposure and duration of exposure of the IgG infection was observed (Table 2). Eighty-seven (31.9 %) of the subjects handled cat litters and a significant statistical association ($p<0.05$) between handling of cat litters and latent infection (IgG) of *Toxoplasma gondii* was found (Table 2).

Majority of the subjects (48.4 %) were found to be grand multiparous, while multiparous and primigravida constitute 45.1 % and 6.6 %, respectively. No significant statistical association ($p>0.05$) as established between gravidity and toxoplasma infection either in the acute (IgM) or the latent (IgG) infection (Table 3). The seroprevalence of toxoplasma IgG was found to be 33.3%, 30.1% and 31.1% for primigravida, multigravida and grand-multigravida while it was found to be 0.0%, 5.7% and 1.5% for IgM in the above order (Table 3). Seroprevalence of anti -

toxoplasma IgM was found to be 5.6%, 4.3%, and 1.6% for first, second and third trimester while it was 35.2%, 25.8%, and 32.5% for IgG (Table 3). One hundred and five subjects had history of still birth. There was no significant statistical association (p>0.05) between still birth and the toxoplasma infection (Table 3). A significant relationship (p>0.05) was found between latent (IgG) form of *T. gondii* infection and history of miscarriage (Table 3).

Table 1: Seroprevalence of Toxoplasmosis According to the Demographic Profile of the Patients

Variable	**IgG**		**IgM**		**P-Value (IgG,IgM)**
	Positive n (%)	**Negative n (%)**	**Positive n (%)**	**Negative n (%)**	
Age (Yrs.)					**0.17,0.40**
11-20	4 (40)	6 (60)	1 (10.0)	9 (90.0)	
21-30	44 (27)	121 (73)	5 (3.0)	160 (97.0)	
31-40	36 (37)	62 (63.3)	3 (3.1)	95 (97)	
Total	84 (31)	189 (69)	9 (3.3)	264 (96.7)	
Religion					**0.46,0.64**
Islam	64 (32)	135 (68)	4 (2.0)	195(98)	
Christianity	20 (27)	54 (73)	5 (7)	69(93.2)	
Total	84 (31)	189 (69)	9 (3.3)	264 (96.7)	
Level of Education					**0.21,0.80**
None	0 (0.0)	1 (100)	0 (0.0)	1 (100)	
Qur'anic	29 (39)	46 (61.3)	4 (5.3)	71 (95)	
Primary	13 (28)	34 (72.3)	1 (2.1)	46 (98)	
Secondary	26 (33)	52 (66.7)	2 (2.6)	76 (97)	
Post-Secondary	16 (22)	56 (77.8)	2 (2.8)	70 (97)	
Total	84 (31)	189 (69)	9 (3.3)	264 (96.7)	
Residence					**0.66,0.26**
Rural	25 (33)	50 (66.7)	4 (5.3)	71 (95)	
Urban	59 (30)	139 (70)	5 (2.5)	193 (97.5)	
Total	84(31)	189 (69)	9 (3.3)	264 (96.7)	

Table 2: Seroprevalence of *T. gondii* infection in Relation to the Risk Factors in the Study Area

Variable	IgG		IgM		P-Value (IgG, IgM)
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	
Contact with Cats					**0.00,0.73**
Yes	62 (60.2)	41 (39.8)	4 (3.9)	99 (96.1)	
No	22 (12.1)	148 (87.1)	5 (2.9)	165 (97.1)	
Total	84 (30.8)	189 (69.2)	9 (3.3)	264 (96.7)	
If Yes, How Many Times					**0.00, 0.19**
Once	11 (91.7)	1 (8.3)	2 (16.7)	10 (83.3)	
Twice	2 (66.7)	1 (33.3)	0 (0.0)	3 (100)	
More Than Twice	51 (58.0)	37 (42.0)	2 (2.3)	86 (97.7)	
Total	64 (62.1)	39 (37.9)	4 (3.9)	99 (96.1)	
Last Contact					**0.00, 0.81**
< 1 Month	58 (62.4)	35 (36.6)	4 (4.3)	89 (95.7)	
1-3 Month	0 (0.0)	3 (100)	3 (100)	0 (0.0)	
> 3 Month	3 (37.5)	5 (62.5)	0 (0.00)	8 (100)	
Total	61 (59.3)	42 (40.7)	7 (6.8)	96 (93.2)	
Handling of Cat Litters					**0.00, 1.00**
Yes	59 (67.8)	28 (32.2)	3 (3.4)	84 (96.6)	
No	25 (13.4)	161 (86.6)	6 (3.2)	180 (96.8)	
Total	84 (30.8)	189 (69.2)	9 (3.3)	264 (96.7)	
Under Cooked Meat					**0.00, 0.18**
Yes	78 (52.7)	70 (47.3)	7 (4.7)	141 (95.3)	
No	6 (4.8)	119 (95.2)	2 (1.6)	123 (98.4)	
Total	84 (30.8)	189 (69.2)	9 (3.3)	264 (96.7)	
Source of Water					**0.66, 0.53**
Pipe Born	52 (30.4)	119 (69.6)	9 (5.3)	162 (94.7)	
Well	32 (32.3)	67 (67.7)	0 (0.0)	99 (100.0)	
Pond	0 (0.0)	3 (100.0)	0 (0.00)	3 (100.0)	
Total	84 (30.8)	189 (69.2)	9 (3.3)	264 (96.7)	

The result of the study revealed that the CD4 cell count of the participants ranged between 17 copies / ml to 1121 copies / ml. The mean CD4 cell count of the subjects is 359.86 copies / ml ± 259.4 copies / ml (Table 4). Seroprevalence of toxoplasma infection in relation to CD4 grouping is higher (72.4%) in those with CD4 cell count of < 200 copies / ml in the case of IgG. However, for IgM the highest prevalence (10.3%) occurred in those with CD4 between 200 to < 350 copies / ml. Significant statistical association ($p>0.05$) was found between *T. gondii* infection and the CD4 cell counts both in IgG and IgM (Table 4).

Table 3: Seroprevalence of *T. gondii* in Relation to the Pregnancy and the Complications of the Infection

Variable	IgG		IgM		P-value (IgG, IgM)
	Positive	Negative	Positive	Negative	
	n (%)	n (%)	n (%)	n (%)	
Gravidity					0.93, 0.18
Primigravida	6 (33.3)	12 (66.7)	0 (0.0)	18 (100.0)	
Multigravida	37 (30.1)	86 (69.9)	7 (5.7)	116 (94.3)	
Grand-multigravida	41 (31.1)	91 (68.9)	2 (1.5)	130 (98.5)	
Total	84 (30.8)	189 (69.2)	9 (3.3)	264 (96.7)	
Trimester					0.40, 0.27
First	19 (35.2)	35 (64.8)	3 (5.6)	51 (94.4)	
Second	24 (25.8)	69 (74.2)	4 (4.3)	89 (95.7)	
Third	41 (32.5)	85 (67.5)	2 (1.6)	124 (98.4)	
Total	84 (30.8)	189 (69.2)	9 (3.3)	264 (96.7)	
History of Still Birth					0.13, 0.09
Yes	38 (36.2)	67 (63.8)	6 (5.7)	99 (94.3)	
No	46 (27.4)	122 (72.6)	3 (1.8)	165 (98.2)	
Total	84 (30.8)	189 (69.2)	9 (3.3)	264 (96.7)	
History of Miscarriage					0.00, 0.17
Yes	77 (49.4)	79 (50.6)	3 (1.9)	153 (98.1)	
No	7 (6.0)	110 (94.0)	6 (5.1)	111 (94.9)	
Total	84 (30.8)	189 (69.2)	9 (3.3)	264 (96.7)	

Table 4: Seroprevalence of *T. gondii Infection* in Relation to the Immunological (CD4 counts) and Full Blood Count Profile

	IgG		IgM		P-value (IgG, IgM)
CD4 Cells/mls	**Positive**	**Negative**	**Positive**	**Negative**	
	n (%)	**n (%)**	**n (%)**	**n (%)**	0.00, 0.04
<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)	
≥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)	

DISCUSSION

This research demonstrates that the prevalence of anti-toxoplasma antibodies among HIV infected pregnant women in Kano state is 34.1%. The chronic or latent form (IgG) of *T. gondii* infection constitutes 30.8% which agrees with that reported from Zaria, Nigeria by Bata *et al.* (2009) where a prevalence of 29.1% for IgG was found. Deji-Agboola *et al.* (2011) in a related study on pregnant women attending antenatal clinic at Federal Medical Center, Lagos, Nigeria reported a prevalence of 32.6% for IgG antibodies. The findings demonstrate high number of circulating antibodies of IgG among the participants which implies low risk of fetal infection. The results also indicate that HIV infected pregnant women could be at higher risks of Sero-conversion from chronic form (IgG) of *T. gondii* infection to the acute form (IgM) which poses a serious consequence especially to the fetus.

The 3.3% IgM prevalence obtained in this study suggests a low prevalence of recent Toxoplasma infection among the pregnant women with more occurring in the first trimester than second trimester and third trimester. The risk of fetal infection is greater in the case of recent toxoplasma infection (IgM) and in the early stage of pregnancy which are seen in this study. The first trimester is the stage of organogenesis and the formation of most important parts of the fetus, hence the likelihood of deformations and even death (Manuel *et al.,* 2020).

The seroprevalence of *toxoplasma gondii* infection was not seen to be increasing with age in this study although not statistically significant, as the highest prevalence was seen with the younger age group of 11-20 (IgG (40%); IgM (10%)) which disagrees with studies by Zemene *et al.* (2012) (88.7% in 14 to 19 years to 55.3% in 30 to 39 years) and Rosso *et al.* (2008) (39.0% in 14 to 19 years to 55.3% in 30 to 39 years) where the prevalence was found to be increasing with age. Toxoplasma seroprevalence was also insignificantly found to be highest in those with lower level of education and reduced as the level of education of the subject increased. Lowest prevalence was seen with those that had post-secondary school level of education and this finding agrees with the report of other researchers who found lower levels of education to be associated with increased risk of Toxoplasmosis (Bata *et al.,* 2009). The observed variation in the prevalence may not be unconnected with the fact that people with higher level of education are more likely to observe good level of hygiene during cooking, playing with pets, consumption of vegetables, fruits and type of water compared to those with lower level of education.

The highest prevalence of toxoplasmosis with regards to the source of drinking water in this study was seen with those using well water, although not statistically significant. This finding agrees with a study done in Zaria, Nigeria by Bata *et al.* (2009) where the highest prevalence in terms of source of water was seen among pregnant women who drank well water. Some of these wells are contaminated and it's a known fact that drinking contaminated water is a source of *T. gondii* infection (Minuzzi *et al.,* 2021).

The seroprevalence of *T. gondii* in terms of residence was found to be insignificantly high among those living in rural areas. This is not surprising as those in the rural areas are not as educated and enlighten as their counterparts in the urban areas which could predispose them to certain risk factors of *Toxoplasma gondii* such as drinking contaminated water, eating contaminated fruits and poorly cooked meat which contribute to the high prevalence of toxoplasmosis (Morais *et al.,* 2021).

Significant association ($p<0.05$) was found between contact with cats; handling of cat litters and the seroprevalence of toxoplasma infection which is similar with findings from Taiwan (Lin *et al.,* 2008) and Ethiopia (Zemene *et al.,* 2012). There is significant statistical association ($p<0.05$) between eating of undercooked meats and toxoplasma antibodies IgG which agrees with a study by Morais *et al.* (2021).

The CD4 cell count is important in the immune status of HIV positive subjects. The higher the CD4 cell count the less the infection with opportunistic pathogens associated with low immunity in AIDS patients of which *Toxoplasma gondii* is implicated. With regards to this study, those subjects with CD4 cell counts of less than 200 cells/mls were seen to have the highest prevalence of toxoplasmosis. The least prevalence was seen in those with CD4 cell count greater than 350 counts. A statistically significant difference ($p<0.05$) was found to exist between *T. gondii* infection and the CD4 cell count in both acute (IgM) and chronic (IgG) infections. This finding agrees with a study carried out by Sadiq (2011) in Kano, Nigeria who also found a statistically significant association ($P < 0.05$) between the seroprevalence of *T. gondii* infection and the CD4 cell counts. Similarly, the findings of this study agree with the report of another study from United States of America by Luft and Remington (1992) where they found a statistical significance difference ($P < 0.05$) between the level of CD4 cell counts and the prevalence of *Toxoplasma gondii* infection. Thus, decline of cellular immunity, exacerbated by pregnancy exposed to the reactivation of latent cysts that predisposed to the risk of trans-placental infection of the fetus may lead to congenital toxoplasmosis. Bachmeyer *et al.* (2006) reiterated that immunosuppression may allow transmission of latent maternal infection to the new born which could be prevented by adequate follow up of maternal HIV and toxoplasma infection during pregnancy.

CONCLUSION

This study has shown that the prevalence of *T. gondii* anti-bodies IgG had the highest prevalence of 30.8% ~~as~~ compared to 3.3% IgM. The results also indicate that HIV infected pregnant women could be at higher risks of Sero-conversion from chronic form (IgG) of *T. gondii* infection to the acute form (IgM) which poses a serious consequence especially to the fetus.

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