






ORIGINAL PAPER

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## Seroprevalence of Toxoplasmosis among Human Immunodeficiency Virus infected pregnant women in Abuja Teaching Hospital, Nigeria

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### ABSTRACT

**Introduction.** Toxoplasmosis is a neglected parasitic infection that has economic and epidemiological significance. Data on toxoplasmosis seroprevalence among Human Immunodeficiency Virus (HIV) infected pregnant women may be of obstetric and neonatal concern.

**Aim.** The study was designed to determine the seroprevalence of Toxoplasmosis and associated risk factors in HIV-positive pregnant women attending the University of Abuja Teaching Hospital, Abuja, Northcentral Nigeria.

**Material and methods.** This was a hospital-based cross-sectional study. A total of 160 HIV seropositive pregnant women were recruited. Blood samples were collected and tested for anti-*T. gondii* IgM and IgG using Enzyme Linked Immunosorbent Assay (ELISA). Structured questionnaires were used to collate the sociodemographic variables of participants.

**Results.** Out of the 160 of HIV seropositive pregnant women, the seroprevalence anti-*T. gondii* IgG and IgM were 29.4% and 4.4%, respectively. There was no significant association between anti-*T. gondii* and all sociodemographic variables studied ( $p>0.05$ ).

**Conclusion.** The overall result of this study revealed that the majority of pregnant women were exposed to toxoplasmosis much earlier in life. Hence, these findings will assist obstetricians and gynecologists in the early diagnosis and management of *Toxoplasma gondii* infection in pregnant women, especially HIV coinfecting ones with IgM seropositivity.

**Keywords.** HIV coinfections, Nigeria, pregnant women, toxoplasmosis

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## Introduction

*Toxoplasma gondii* (*T.gondii*) is regarded as one of the causes of opportunistic parasitic diseases amongst HIV infected persons, especially pregnant women. Severe morbidities can occur to both mother and fetus.<sup>1</sup> Cell-mediated immunity, which typically develops after acute infection with *T. gondii*, does not eradicate the disease. The expression of IgM and IgG antibodies in sera is indicative of recent or primary and past exposure to *T. gondii*, respectively.<sup>1</sup>

Primary toxoplasmosis in pregnant women can have serious consequences for the mother and fetus.<sup>1</sup> Although the mortality rate of this parasitic infection in adults appears very low, it causes devastating effects including blindness, neurological impairment, and mental retardation in children when infected in utero with worse consequences in children of immuno-compromised mothers.<sup>1,2</sup>

In Brazil, a recent report shows that 63% of pregnant women were positive for IgG and 5.3% for IgM against *T. gondii*.<sup>3</sup> Tanzania, Ethiopia, and Ghana have reported rates of 44.5%, 83.6%, and 92.5%, respectively, among the studied pregnant population.<sup>4-6</sup> Prevalence of IgG and IgM specific antibodies among pregnant women in Egypt was 38.3% and 18.3%, while a rate of 39.9% and 2.5% was reported in Turkey, respectively.<sup>7,8</sup> A report from Sokoto-Nigeria showed that variations across trimesters with 30.5%, 22.5%, and 30.7% in the first, second, and third trimesters of pregnancy respectively.<sup>9</sup>

In immuno-competent mothers, exposure to *T. gondii* before pregnancy carries virtually no risk of fetal infection; this predominantly occurs in primary infections acquired while pregnant.<sup>10</sup> The immuno-compromised state as seen in HIV positive pregnant women, however, tends to facilitate in-utero vertical transmission. This is mostly as a consequence of latent infection or reactivation.<sup>11-13</sup> In patients with HIV/AIDS, reactivation can occur with CD4+ T cell count of <200 cells/ $\mu$ L.<sup>14</sup> When the CD4+ T cell count is less than 100 cells/ $\mu$ L, the clinical and symptomatic toxoplasmosis becomes very likely.<sup>14,15</sup> Reactivation typically manifests as central nervous system involvement.<sup>15</sup>

The disease severity, however, decreases with gestational age with first-trimester infection resulting in major sequelae or pregnancy loss and mild or no involvement apparent at birth.<sup>16</sup> Secondary prevention is by serological screening to identify those that acquire an infection during pregnancy, and if the fetal infection is detected, therapeutic options including termination of pregnancy and antibiotic treatment to the fetus in utero should be discussed.<sup>17</sup> Despite the relatively high seroprevalence of HIV infection and increased risk of toxoplasmosis in women, there is a paucity of toxoplasmosis studies in HIV infected pregnant women in Northern Nigeria.

## Aim

Hence, this study was designed to determine the seroprevalence of Toxoplasmosis and associated risk factors in HIV-positive pregnant women attending the University of Abuja Teaching Hospital, Abuja, Northcentral Nigeria.

## Material and methods

### Study Design

This was a hospital-based cross-sectional study conducted from July 2013 to August 2014.

### Study Area

The study was conducted at the Departments of Obstetrics and Gynaecology and Immunology, University of Abuja Teaching Hospital, Abuja. Women were enrolled at ante-natal clinics of the University of Abuja Teaching Hospital (UATH), Gwagwalada, Abuja, Nigeria. The hospital provides health care services to the inhabitants of Abuja and neighboring states including Niger, Kaduna, Kogi, and Nassarawa states. The Hospital has an average of 3,000 deliveries annually.

### Study Population

This included consecutively enrolled 160 HIV-positive pregnant women who provided signed informed consent prior to enrolment. After signing informed consent, each participant completed a structured questionnaire on sociodemographic characteristics.

### Inclusion Criteria

HIV-infected pregnant women between the ages 15year to 45 years attending the UATH.

### Exclusion Criteria

1. Persons who did not consent to be part of the study.
2. Pregnant women less than 15 years.
3. Non-pregnant women
4. Those not on antiretroviral therapy

### Study Sample Size

Earlier studies in the geo-political zone of the study location found that the seroprevalence of toxoplasma IgG antibodies in pregnant women was 2.4%.<sup>[18]</sup> Therefore, the minimum sample size at 95% level of confidence interval was calculated using Fischer's formula for cross-sectional studies. The calculated minimum sample size was 40. However, this was increased by 4 folds to 160 to enhance the statistical credence of the study.

### Ethical Approval

The ethical clearance for this study was obtained from the Research Ethics Committee of the University of Abuja Teaching Hospital (Approval Number: FCT/UATH/HREC/PR/416). Written informed consent and

accent (as the case may be) were sought from all participants before enrollment into the study. All data were treated with utmost confidentiality and anonymized throughout the study.

#### Sampling Method and Data Collection

Subjects were enrolled randomly. Interviewer-administered, structured questionnaires were used to collate sociodemographic data of subjects. The questions outlined in the data forms were explained to the subjects and then completed with the required information which included biodemographic data.

Each client that consented was requested to sit on a chair comfortably and was informed that she might experience a little discomfort during the venepuncture. The site intended for the venepuncture at the forearm was cleansed with an alcohol swab, and a selected vein from the forearm was pricked with a sterile needle attached to a Vacutainer bottle and 5 ml of blood was drawn. The needle was gently withdrawn and dry cotton was applied on to the site with a gentle pressure applied to achieve hemostasis. The entire procedure was aimed to be of minimal risk to the clients. Each needle was used only once and properly discarded after use into a sharp container. This procedure was done by the researcher and two research assistants and dispensed into a labeled 10ml plain bottle. These were put in racks and transported to the laboratory. The serum was separated by centrifugation at 10000xg for 10 minutes at 18°C. After centrifugation, the separated sera were refrigerated (2-8°C) until analysis was done within 24 hours of sample collection. The serum was carefully removed using a fine bore pipette to avoid extracting red cells. The samples were analyzed for the presence of immunoglobulin G or M (IgG/IgM) class antibodies to *T. gondii* Enzyme-Linked Immunosorbent Assay (ELISA) using Toxo IgG and IgM ELISA kit from Fortress Diagnostics Limited, (North Ireland, UK)

The fortress diagnostics® (TOXO IgG/IgM) ELISA kits are qualitative immunoassays for the detection of human antibodies in serum or plasma directed against *T. gondii*. The sensitivity and specificity of the Toxoplasma IgG and IgM ELISA kits were both 99.9% on plasma and sera. The test was done and the results interpreted following kits manufacturer's instructions. Multiskan™ FC Microplate Photometer (Thermofischer Scientific Inc., Massachusetts, United States) was used to measure the absorbance of the final reaction products of the enzyme immunoassay.

#### Statistical Analysis

All generated data were analyzed using Statistical Package for Social Science (SPSS) (California Inc, USA) version 24. Association between the seroprevalence of *T. gondii* antibodies and sociodemographic variables of

pregnant women was determined by two-tailed Chi-square test. *P* values <0.05 at 95% confidence interval were reported as statistically significant.

#### Results

This study was carried out among 160 HIV seropositive pregnant women between the ages of fifteen years and forty-five years and none withdrew after consenting to the study. The mean age of HIV seropositive pregnant women was 30.4±2.2 with the highest proportion within the age range of 20-39 years accounting for 55.7% of the pregnant women enrolled and the lowest proportion being 40-45 years group accounting for 20.0%. However, this distribution was not statistically significant (*p*>0.05, Table 1). The mean gestational age and mean parity of the total study population were 28.7±3.1 and 3.4±1.3 years, respectively.

Out of the 160 HIV seropositive pregnant women whose blood was examined for the presence of *T. gondii* antibodies, 18 of these subjects were positive for anti-Toxoplasma antibodies with the overall seroprevalence of *T. gondii* antibodies was 33.8%. The seroprevalence of anti- *T. gondii* IgG and IgM were 29.4% and 4.4%, respectively.

Anti-*T. gondii* IgG was predominantly obtained among 20-39 years age group, representing 95.7% of the total IgG anti-Toxoplasma antibodies. None was obtained from the age group <19 years. From the 20-39 years age group, 7 blood samples analyzed were positive for IgM anti-Toxoplasma antibodies, representing 100% of the total IgM anti-Toxoplasma seropositive in the study. The distribution of IgG and IgM anti-*T. gondii* antibodies among HIV-positive pregnant women was not statistically significant (*p*=0.778 and 0.804 for IgG and IgM, respectively) (Table 1).

From the 160 HIV seropositive pregnant women, 56 women were nulliparous, 54 with parity between 1-4, and 50 women with parity of five and above. 22 (46.8%) anti-*T. gondii* IgG was obtained among women with 1-4 parity and 19 women representing 40.4% with anti-*T. gondii* IgG were obtained from women with >5 parity. However, anti-*T. gondii* IgM was predominantly obtained among pregnant women with parity of 1-4, representing 57.1% of the total anti-*T. gondii* IgM. The distribution of IgG and IgM anti-*T. gondii* antibodies regarding parity of the pregnant women was not statistically significant (*p*=0.642 and 0.909 for IgG and IgM respectively, Table 1).

Out of the 160 of HIV seropositive pregnant women, anti-*T. gondii* IgG were predominantly obtained among pregnant women in the third trimester with 48.9% of the total anti-*T. gondii* IgG positive and 44.3% of those with anti-*T. gondii* IgG negative results (Table 1). Anti-*T. gondii* IgM was predominantly obtained among pregnant women in the third trimester with

**Table 1.** Sociodemographic characteristics and *Toxoplasma gondii* antibodies among HIV seropositive pregnant women\*

Variable	IgG		P value	IgM		P value
	Positive (%)	Negative (%)		Positive (%)	Negative (%)	
Age (years)						
≤ 19	0(0.0)	5(4.4)	0.778	0(0.0)	5(3.3)	0.804
20–39	45(95.7)	98(86.7)		7(100.0)	136(88.9)	
≥ 40	2(4.3)	10(8.9)		0(0.0)	12(7.8)	
Total	47(29.4)	113(70.6)		7(4.4)	153(95.6)	
Place of residence						
Urban	43(91.5)	84(74.3)	0.360	7(100.0)	120(78.4)	0.790
Rural	4(8.5)	29(25.7)		0(0.0)	33(21.6)	
Total	47(29.4)	113(70.6)		7(4.4)	153(95.6)	
Educational status						
No formal education	3(6.4)	25(70.6)	0.061	0(0.0)	28(18.3)	0.248
Primary	14(29.8)	9(8.0)		2(28.6)	21(13.7)	
Secondary	10(21.3)	38(33.6)		0(0.0)	48(31.4)	
Tertiary	20(42.6)	41(36.3)		5(71.4)	56(36.6)	
Total	47(29.4)	113(70.6)		7(4.4)	153(95.6)	
Parity						
Nulliparous	6(12.8)	50(44.3)	0.642	1(14.3)	55(35.9)	0.919
Para 1-4	22(46.8)	32(28.3)		4(57.1)	50(32.7)	
≥ Para 5	19(40.4)	31(27.4)		2(28.6)	48(31.4)	
Total	47(29.4)	113(70.6)		7(4.4)	153(95.6)	
Gestational age						
1 <sup>st</sup> Trimester	9(19.2)	25(22.1)	0.606	1(14.2)	33(21.6)	0.119
2 <sup>nd</sup> Trimester	15(31.9)	38(33.6)		3(42.9)	50(32.7)	
3 <sup>rd</sup> Trimester	23(48.9)	50(44.3)		3(42.9)	70(45.7)	
Total	47(29.4)	113(70.6)		7(4.4)	153(95.6)	

\*Significant association determined by two-tailed Chi-square test

48.9% of the total anti-*T. gondii* IgG positive and 44.3% of those with anti-*T. gondii* IgG negative results from 20-39 years age group, 7 blood samples analyzed were positive for anti-*T. gondii* IgM. Subjects between 20-39 year age group represented 95.7% of the total anti-*T. gondii* IgG seropositives. None was obtained from the age group <19 years. For subjects within the 20-39 years age group, 7 of the blood samples analyzed were anti-*T. gondii* IgG seropositive.

## Discussion

In this study, the seroprevalence of anti-*T. gondii* IgG and IgM were 29.4% and 4.4%, respectively, with an overall seroprevalence of 33.8%. This is closely comparable with 27.4% and 34% recorded from similar studies reported at Ibadan and Kano cities of Nigeria, respectively.<sup>18,19</sup> The relatively high prevalence recorded in this study indicates that pregnant women living with HIV/AIDS may be susceptible to repeated exposure or reactivation of chronic *T. gondii* infection in our setting. The overall prevalence of *T. gondii* antibodies in our study was higher than 29.9% recorded in Zaria but lower than

others carried out in Lagos and Maiduguri where 40.2% and 48.9% were recorded respectively.<sup>1,20,21</sup> Lagos is a bustling riverine area where outdoor activities thrive while Maiduguri dwellers have been ravaged by insecurity that has grossly affected their standard of living including access to potable water, hygienic meats, and vegetables.

Studies reported within and outside Africa have recorded very high seroprevalence of toxoplasmosis of 83.6%, 92.5%, 48.0%, and 63.0% among pregnant women in Ethiopia, Ghana, India, and Brazil, respectively.<sup>3,5,6,22</sup> Perhaps, geographic and hot climatic conditions in these countries that favor sporulation would have accounted for these wide margins in prevalence.<sup>10,14,23-25</sup> The climatic condition of our study area may not be as favorable as those listed above for oocysts' survival. Conversely, much lower rates (less than 10%) have been reported in the USA and UK.<sup>26,27</sup> It is expected that these high-income nations had good waste disposal systems, potable water, and high standards of living, in addition to a temperate climate that will not favor the thriving of *T. gondii* oocysts.

In this study, there were no significant associations among the socio-demographic characteristics. Hence, this is a negative result. However increasing age, tertiary education, and urban settlement appear to facilitate infection.<sup>28</sup> Age as a factor may be an indicator of high susceptibility of exposure to many risk factors for primary *T. gondii* infection during their lives than younger individuals.<sup>29</sup> Such findings have also been observed by Ogoina *et al* and Al-Harathi *et al* in Zaria and Saudi Arabia respectively.<sup>30,31</sup> Gwagwalada, a suburb in the federal capital of Nigeria, has also accommodated an increasing number of urban dwellers from various educational and social backgrounds which may have contributed to these socio-demographics. These findings are also in agreement with reports from most studies around the world.<sup>1,5,21,31</sup>

## Conclusion

The overall result of this study revealed that the majority of pregnant women were exposed to toxoplasmosis much earlier in life. Hence, these findings will assist obstetricians and gynecologists in the early diagnosis and management of *Toxoplasma gondii* infection in pregnant women, especially HIV coinfecting ones with IgM seropositivity.

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