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# SEROPREVALENCE OF IgG ANTI- *T. GONDII* ANTIBODY AMONG HIV-INFECTED PATIENTS IN MAIDUGURI, NORTH EASTERN NIGERIA.

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**Abstract**

**Background**: Toxoplasma gondii infection is one of the commonest opportunistic infections in HIV-infected patients, with the fatal consequences of toxoplasmic encephalitis particularly in advanced disease. However, data regarding T.gondii infection in the setting of HIV/AIDS are scant in Nigeria. **Objective**: To determine the seroprevalence of T.gondii amongst HIV-infected patients as well as to determine the correlation between anti-T.gondii IgG titre and the CD4+ cell count/HIV-1 RNA viral load. **Method:** A cross sectional study in which a total of 190 subjects were involved i.e. 110 newly diagnosed HAART naïve HIV-positive patients and 80 apparently healthy HIV-negative age- and-sex matched controls that were selected by simple random sampling method. **Results:** The age range of the study population was 20-64 years. The mean ages of male subjects for both HIV-positives and controls were 37.52 ±8.20 years and 35.79 ±12.31years, respectively, (p= 0.462). On the other hand, the mean ages of female subjects for both HIV-positives and controls were 29.90 ±6.98 years and 32.30 ±10.29 years, respectively, (p=0.149). Twenty one subjects (19.1%) among HIV-positives and 1 (1.25%) HIV-negative tested positive for anti-T.gondii IgG, respectively, (p= 0.000). The prevalence rate ration of anti-T. gondii IgG of HIV positives compared to HIV-negatives was 15.28. Significant proportion of anti-T.gondii positive subjects presented with AIDS defining illnesses compared with their anti-T.gondii negative counterparts. **Conclusion:** The study has shown that anti-T.gondii IgG is about 15 times more prevalent among HIV positive patients compared to controls. Routine screening for T.gondii IgG anti-body is therefore recommended for all HIV-infected subjects at the facility as well as commencement of chemoprophylaxis against Toxoplasmic encephalitis in HIV-infected patients with CD4+ cell count of <100 cells/ml.

**Keyword:** *Toxoplasma gondii*, IgG antibody, CD4+ cell count, HIV-1 Viral RNA viral load.

## Introduction

Toxoplasmosis is a zoonotic disease caused by the obligate intracellular coccidian protozoa Toxoplasma gondii (T. gondii). It has been recognized as a major cause of morbidity and mortality especially among patients with advanced Human immunodeficiency virus (HIV) disease.[1] Toxoplasma gondii infects the nucleated cells of virtually all warm-blooded

animals making it one of the most successful protozoan parasites. It is a frequent cause of sub-clinical latent human infection and occurs in about half of the world's population though mostly asymptomatic.[1, 2] The definitive host is cat and the infection is acquired by oral ingestion of raw or undercooked meat (tissue cyst) and cat excreta (oocyst). The first contact with T. gondii usually cause no clinical symptoms in subjects with efficient immune system, but the protozoan may survive in the form of a cyst in the central nervous system(CNS), heart and skeletal muscles.[1-4]

Reactivation of chronic CNS infection with resultant toxoplasmic encephalitis (TE) occurs almost exclusively in patients with Acquired Immunodeficiency Syndrome (AIDS), or those with defects in cell mediated immunity.[4, 5, 6] Varying anti-T. gondii antibody seroprevalence levels among HIV and non-HIV patients have been reported from different parts of the world.

## Materials And Method

***Study design:*** The study was a cross sectional comparative study.

***Study population:*** Adult patients ≥15 years of age diagnosed to have HIV infection formed the study group. Eighty apparently healthy age- and-sexes matched HIV-negative individuals served as controls.

***Selection criteria:*** all consenting newly diagnosed HIV-positive patients ≥15 years of age.

***Exclusion criteria:*** **n**on consent, persons younger than 15 years of age, patients on immunosuppressive drug therapy, patients with diabetes mellitus, chronic renal failure or widespread malignancies.

***Nature of specimen:*** Five millilitres (5ml) of blood sample was obtained from each subject by venepuncture after cleansing the site with methylated spirit. This was placed in plain tubes and allowed to clot. The sera was separated from the cells, frozen at – 20$^0$C to be tested within 72 hours.

### *IgG ANTI-TOXOPLASMA GONDII ANTIBODY (IgG) SCREENING*

The enzyme-linked immunosorbent assay (ELISA) method was used to detect T .gondii specific IgG, which depends on the ability of T. gondii tachyzoite antigen to be bound by human anti-T.gondii specific IgG. Toxoplasma-specific IgG serological test was performed on patients' blood samples using an ELISA kit employing a tachyzoite antigen extract. The standard ELISA commercial kit (AxSYM, Abbott laboratory Abbott Park, Illinois, USA) was used in accordance with manufacturer's instructions.

***ETHICAL CONSIDERATION:*** Ethical approval for the study was obtained from the Ethical Committee of the University of Maiduguri Teaching Hospital. A signed or thumb-printed informed consent was obtained from all participating subjects before commencing the study.

### Data Analysis

The data was manually entered into a computer data base and was subsequently analysed using SPSS version 16.0 (SPSS, Chicago, Ill, USA). Values were expressed as mean ± Standard Deviation (M±SD). Student t-test (with Yates correction) and Chi-square were used to test significance of differences between continuous variables and proportions respectively. A p value of $<0.05$ was considered significant.

## Results

A total of 190 subjects were included in the study comprising 110 newly diagnosed HIV-positive HAART-naive patients and 80 apparently healthy age- and-sex matched HIV-negative controls. Amongst the 110 HIV-positive subjects; 40 were males and 70 females, (male to female ratio = 1:1.8). Amongst the 80 controls, there were 40 male and 40 female subjects, respectively, (male to female ratio = 1:1). The mean ages of male subjects among

cases and controls, respectively, were; 37.52 ±8.20 years and 35.79±12.31years, (p= 0.462). On the other hand, the mean ages of female subjects among cases and controls were; 29.90 ±6.98 years and 32.30 ±10.29 years, respectively, (p=0. 149).

Twenty-one subjects among the HIV-positive subjects were anti-T.gondii IgG positive giving a prevalence of 19.1%, whereas only one subject (1.25%) was positive among HIV negative controls. There is a statistically significant difference in the seroprevalence rates between the two groups (p = 0.000). The prevalence rate ratio of HIV-positives compared to HIV-negative controls was 15.28.

The mean CD4+ cell count of anti-T.gondii positive subjects was 173.24 ±151.98 cells/µl and that of negative ones was 222.15 ±186.14 cells/µl, respectively. There was no statistically significant difference between the mean CD4+ cell counts of the two groups (p = 0.265). Similarly, the mean HIV-1 RNA viral load of anti-T.gondii positive subjects was 97,454.56 ±16,045.50 copies/ml, while that of negative ones was 81,386.67 ±84,040.81 copies/ml, respectively, (p = 0.658).

***TABLE 1:* Age-Group Distribution of Cases And Controls**

| Age-group (years) | Cases N (%) | Controls N (%) | P value |
|---|---|---|---|
| 20-24 | 11 (10.0) | 15 (18.75) | 0.129 |
| 25-29 | 23 (20.9) | 18 (22.5) | 0.931 |
| 30-34 | 19 (17.3) | 15 (18.75) | 0.948 |
| 35-39 | 23 (20.9) | 8 (10.0) | 0.070 |
| 40-44 | 12 (10.9) | 11 (13.8) | 0.704 |
| 45-49 | 12 (10.9) | 5 (6.2) | 0.387 |
| 50-54 | 7(6.4) | 3 (3.8) | 0.696 |
| 55-59 | 1 (0.9) | 3 (3.8) | 0.390 |
| 60-64 | 2 (1.8) | 2 (2.5) | 1.000 |
| **TOTAL** | **110 (100)** | **80 (100)** | |

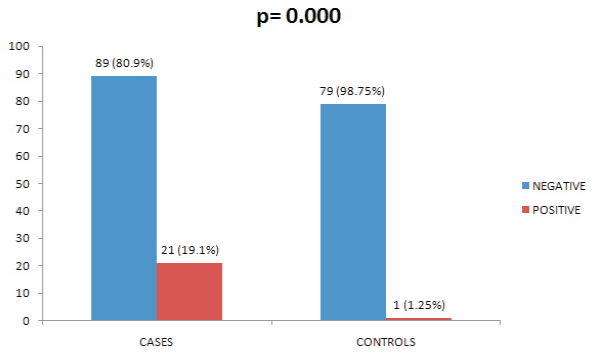


***Table 2:* Improved Who Clinical Staging of Anti-T.gondii Positive And Anti-T.gondii Negative Subjects**

| CD4+ cell count (cells/µl | T.gondii positive WHO CLINICAL STAGE N (%) | | | | T.gondii negative WHO CLINICAL STAGE N (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 1 | Stage 2 | Stage 3 | Stage 4 |
| A (≥ 500) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 3 (3.37) | 2 (2.25) | 2 (2.25) | 0 (0.00) |
| B (200-500) | 1 (4.76) | 0 (0.00) | 2 (9.52) | 3 (14.23) | 15 (16.85) | 7 (7.87) | 8 (8.99) | 3 (3.37) |
| C (< 200) | 2 (9.52) | 3 (14.23) | 4 (19.05) | 6 (28.57) | 18 (20.22) | 13 (14.61) | 10 (11.24) | 8 (8.99) |
| TOTAL | 21 (100) | | | | 89 (100) | | | |

## Discussion

Sub-Saharan Africa remains the epicentre of the global AIDS pandemic.[7] The region has just over 10% of the world's population, but is home to more than 60% of all people living with HIV (22.4 million).[7] In 2007, an estimated 2.5 million people in the region became newly infected, while 2.1 million adults and children died of AIDS.[7]

Nigeria, being the most populous country on the African continent will continue to remain vulnerable to the threats of global pandemics like HIV/AIDS. Chronic opportunistic infections like tuberculosis, Pneumocystic Jirovecii pneumonia, toxoplasmic encephalitis e.t.c. are among the leading cause of morbidity and mortality especially in advanced HIV/AIDS.[7] The anti-T.gondii IgG antibody seroprevalence rate of 19.1% with a prevalence rate ratio of 15.28 among the sub-population of HIV-infected which is significant compared to HIV-negatives, corroborates the fact that toxoplasmosis is a major threat to HIV/AIDS patients in Nigeria (considering the consequences associated with toxoplasmic encephalitis in the setting of HIV infection), as reported in other parts of the world.[7-8,] The

reason for the difference in T.gondii IgG seroprevalence rate between HIV-positive patients and controls in this study is not immediately clear. However, the controls were recruited from voluntary blood donors and ante-natal clinic attendees who were predominantly resident in Maiduguri and its environs i.e. an urban or peri-urban setting. This factor can influence the prevalence rate of T.gondii infection because of variation in the distribution of epidemiologic risk factors responsible for the transmission of T.gondii, personal hygiene, environmental sanitation, as well as access to health information, education and communication compared to the sub-populations of HIV-positives who may be substantially from wide and far flung areas .[47,12]

[9]

The finding of anti-T.gondii IgG seroprevalence rate of 19.1% among HIV-infected subjects favourably compares with figures reported in studies elsewhere i.e. both within and outside Africa e.g. Leport C et al[9] and Nissapotorn V et al[10-12] found a seroprevalence rate of 15-37%, 21% and 22.4% amongst HIV-positive patients in France, Malaysia, and Thailand, respectively. In similar studies done in Africa; Simpore et al[13], Maiga et al[14], Julvez et al[15], and Zumla et al[16] reported anti-T.gondii IgG seroprevalence rates of 25.3%, 21.0%, 18.0%, and 27.0% amongst HIV-infected patients in Burkina Faso, Mali, Niger Republic, and Uganda, respectively. However, the seroprevalence rate of 19.1% is substantially lower than rates reported by Del Rio et al[17] (50%) and Falasi et al[18] (56%), amongst hospitalised HIV-positive patients in Mexico and Spain, respectively. Meisheri et al[8] reported a significantly much higher anti-T.gondii IgG seroprevalence rate of 69.0% amongst HIV-infected patients in Mumbai, India. Conversely, the rate is significantly higher than those reported by Toshio et al[19,] (5.4%), and Hari et al[20,] (8.0%) in Japan and South Africa, respectively. Similarly, Uneke et al[21,] in Jos, North Central Nigeria, reported an anti-T.gondii IgG seroprevalence rate of 38.8% amongst HIV-infected adults. The factors driving these regional and continental differences are unclear. However, these cross-study variations may be because of differences in the sensitivity of various anti-T.gondii detection techniques, as well as variation in the distribution of geo-epidemiologic risk factors responsible for the acquisition of T.gondii infection amongst the various study populations.

On the other hand, the seroprevalence rate of 1.25% amongst apparently healthy controls was by far lower than most reported rates elsewhere e.g. Sundar and colleagues[22] reported an anti-T.gondii IgG seroprevalence rate of 20.3% among healthy voluntary blood donors in Karnataka, Western India. During the 4th National Health and Nutritional Examination Survey (NHNES) by the CDC in the USA (1999-2000), anti-T.gondii IgG seroprevalence rate of 15.8% was reported amongst apparently healthy general population of the US.[23] From South America, in an earlier survey at five blood banks in Natal-Rio Grande de Norte, Brazil, in a sample of 183 donors (average age 21-30 yr), 43.7% had antibody to toxoplasma.[24] At North London Blood Transfusion Centre (NLBTC), when 392 apheresis donors providing granulocyte concentrate were screened, 36% were anti-T.gondii IgG seropositive.[25] These samples were screened to provide a panel of blood donors negative for toxoplasma antibody for seronegative recipients under exacting screening conditions. From Czech Republic among 3758 blood donors, nearly 80% had low titres and 10.96% high titres of IgG antibody to toxoplasma.[26] At Asir General Hospital, a tertiary care hospital in South Western part of Saudi Arabia, on screening 1000 apparently healthy donors, a prevalence of 52.1% to toxoplasma seropositivity was found.[27] Studies across Africa show regional variability in the distribution of anti-T.gondii IgG

seroprevalence rates among the general populations of the various regions e.g. Gueber-Xabier et al[28], Bouratbine et al[29], Adou-Bryn et al[30], Faye et al[31], Bowny et al[32], Doehring et al[33,], and El-nahas et al[34] reported anti-T.gondii seroprevalence rates of 75.0%, 58.0%, 53.6%, 40.2%, 35%, 35%, and 34.1% in Ethiopia, Tunisia, Benin Republic, Senegal, Cote d Ivoire, Tanzania, and Sudan, respectively.

Seroprevalence rates in the general population in India vary from a low rate of 1%-11% to as high as 57%.[35-40] The seroprevalence rate reported from urban and rural samples from Chandigarh was 4.7%,[38] while in hospital based samples from Jodhpur in Rajasthan was 17.2 %.[39] From Mumbai, Meisheri *et al*[7] found a much higher seroprevalence (30.9%) in the general population, with a mean titre of 3.52 IU/ml for toxoplasma antibody. These variations could be related to socio-cultural habits, geographic and environmental factors, the state of general hygiene in the society and the routes of transmission.

Conversely, Onadeko and colleagues[41] in Ibadan, reported a much higher seroprevalence rate among pregnant women attending ante-natal clinic at the University College Hospital (UCH). The reason for the comparably higher rate in Ibadan could be due to the fact that the UCH serves as a referral centre to many population settlements around Ibadan and its environs (i.e. both Urban and rural settings). Therefore, there could be variation in terms of distribution of risk factors for the acquisition of T.gondii infection as well as differences in socio-economic factors amongst these diverse sub-populations.[42-44] Additionally, quite a substantial proportion of the control subjects in this study were from voluntary blood donors most of whom were enlightened individuals such as university undergraduates' etc belonging to that age group.

**Conclusion**

The study has shown that the seroprevalence of Toxoplasma gondii IgG antibody among HIV-infected patients is about 15 times higher that HIV-negatives controls at the facility. In addition, anti-T.gondii positive subjects have been observed to have presented with more AIDS defining clinical conditions and a relatively low mean CD4+ cell count and a higher mean HIV-1 RNA viral load compared to HIV-negative subjects.

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