

Effect of CD4 Count on the Prevalence of IgG and IgM Anti-Toxoplasma Antibodies Among HIV Positive Patients in Benin City, Edo State, Nigeria.

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ABSTRACT

Objectives: Toxoplasmosis is a zoonotic infection with worldwide distribution that can have fatal outcomes among HIV/AIDS patients. This study was aimed at determining the prevalence of IgG and IgM antibodies to *T. gondii* among HIV positive patients as well as their relationship with CD4 count.

Methods: Blood specimens were collected from 1500 subjects consisting of 1200 HIV positive patients and 300 apparently healthy non-HIV subjects. The HIV patients comprised 936 on anti-retroviral therapy (Highly Active Antiretroviral Therapy) (HAART) and 264 HAART-naïve HIV patients. The blood specimens were used to determine CD4 counts using flow cytometry as well as the presence of IgG and IgM anti-Toxoplasma antibodies using Immunochromatographic kit **Results:** Of the 1500 blood specimens, 329 (21.93%) had antibodies to *T. gondii* (11.13% and 7.53% for IgG and IgM respectively; and 3.27% for both IgG and IgM antibodies). Generally, and among HIV patients on HAART ($p<0.0001$) and non-HIV subjects ($p=0.0890$) the prevalence of IgG antibodies was higher, while among HAART-naïve HIV patients ($p=0.0152$) the prevalence IgM antibodies to *T. gondii* was higher. Irrespective of treatment status, the prevalence of antibodies to *T. gondii* was significantly higher among patients with CD4 count <200 cells/ μ L compared with those with CD4 count ≥ 200 cells/ μ L ($p<0.01$). Among HAART-naïve HIV patients and those on HAART, the prevalence of anti-*T. gondii* IgM were significantly higher among patients with CD4 count <200 cells/ μ L ($p<0.05$) while IgG antibodies to *T. gondii* were higher in patients with CD4 count ≥ 200 cells/ μ L ($p<0.05$).

Conclusion: Acute toxoplasmosis among HIV patients was associated with immunosuppression. Measures to prevent *T. gondii* infection and its associated sequelae are advocated.

Key words: HIV, Toxoplasmosis, Immunosuppression, Antibodies.

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INTRODUCTION

Toxoplasma gondii (*T. gondii*) as a ubiquitous parasite is one of the most common zoonotic parasitic diseases in almost all warm-blooded vertebrates across the world (1). *Toxoplasma* infection affects about one-third of the world's population, but most infected individuals remain asymptomatic (2). In man, *Toxoplasma* infection is acquired mainly by ingestion of tissue cysts of the parasite in raw or undercooked meat, by ingestion of parasite oocysts in cat faeces that contaminate soil, vegetables and other food sources, and trans-placentally from infected mothers to their infants (2, 3).

In healthy humans, the infection with *T. gondii* is usually asymptomatic, but it can be fatal in the immunocompromised individuals, such as HIV/AIDS patients, cancer patients, and organ transplant recipients (4-7). Toxoplasmosis of immunosuppressed individuals is most often the result of reactivation of latent infection, though acute acquired *T. gondii* infection may also occur and involve multiple organs (8). Toxoplasmosis is an opportunistic infection that is more prevalent among immunocompromised persons (9). The association of CD4 counts with *T. gondii* infection shows conflicting reports. Some authors did not find any significant association between CD4 count and the prevalence of *T. gondii* infection (10, 11). Others have reported that HIV patients with CD4 cell counts <200cells/ μ l were more likely to be *Toxoplasma* seropositive than those with counts >200cells/ μ l (12, 13). There is paucity of reports on the association of CD4 count and seroprevalence of Toxoplasmosis among HIV positive patients

in Benin City, Nigeria. Thus, this study is aimed at determining the prevalence of IgG and IgM antibodies to *T. gondii* among HIV positive patients as well as the relationship between these antibody types and CD4 count

MATERIALS AND METHODS

Study Population

This study was carried out in Central Hospital Benin City, Nigeria - a secondary healthcare institution that caters for HIV infected persons. A total of 1500 participants were recruited for this study. The study participants consisted of 1200 HIV positive patients (936 On HAART and 264 HAART-naïve) and 300 apparently healthy HIV seronegative individuals which served as controls. The HIV patients attended HIV clinics in the hospital while the non-HIV subjects were attending HIV voluntary counseling centre in the hospital. Informed consent was obtained from all subjects or their parents/guardians in cases of children prior to specimen collection. This study was approved by the Ethical Committee of Edo State Ministry of Health, Benin City (HM.1208./102).

Collection and Processing of specimen

Five milliliters of blood was collected from each participant and dispensed into ethylene diamine tetra acetic acid (EDTA) container, mixed and labelled. The blood specimens were used for CD4 count determination and detection of *T. gondii* antibodies (IgG and IgM)

CD4 Estimation

Blood samples were analysed for CD4-T-lymphocyte count using flow cytometry (Partec, GmbH, Germany) following the manufacturer's instruction. Briefly, 20 μ L of

CD4 phycoerythrine antibody was placed into a Partec test tube and 20 μ L of well mixed whole EDTA blood specimen was added. The content was mixed gently and allowed to stand in the dark for 15 minutes at room temperature. This mixture was agitated every 5 minutes. Eight hundred microlitres of CD4 buffer solution was added to the mixture of antibody and blood specimen and mixed gently. This was then inserted to the counter for counting.

Detection of *Toxoplasma gondii* antibodies

Antibodies to *Toxoplasma gondii* were detected from plasma samples of each subject using an immunochromatographic test kit – One Step Anti-*Toxoplasma gondii*-IgG/IgM test (Diagnos, China) following the manufacturer's instruction. Briefly, 100 μ L of plasma was added to the specimen area of the test kit. This was allowed to flow by capillary action and the results read after 15 minutes. A red or pink line appearing only in the control area indicates negative result. A red line appearing in the control and IgG areas only indicates positive for only IgG antibodies. A red line appearing in both the control and IgM areas indicates positive for IgM antibodies only while three red lines appearing in the control, IgG and IgM areas indicated positive for both IgG and IgM antibodies.

Statistical Analysis

The data obtained were analyzed with Chi square test using the statistical software INSTANT (Graph Pad Inc., La Jolla, CA, USA). A p value of <0.05 was taken as significant.

RESULTS

The seroprevalence of *Toxoplasma gondii* in this study was 21.93% (329 out of 1500). Of this, 11.13% had IgG antibodies only to *Toxoplasma gondii* while 7.53% had IgM antibodies and 3.27% had both IgG and IgM antibodies to *T. gondii* (Table 1). Generally, and among non-HIV subjects as well as HIV patients on HAART, the prevalence of IgG antibodies was higher (general and HIV patients on HAART, $P < 0.001$ each) but failed to reach statistical significance among non-HIV subjects ($p=0.0890$). However among HAART-naïve HIV patients, the prevalence of IgM antibodies (17.69%) was significantly higher ($p= 0.0152$) compared with the presence of IgG (12.12%) and a combination of IgG and IgM antibodies (9.09 %) (Table 1).

With the exception of *T. gondii* IgG antibodies among HAART-naïve HIV patients, the prevalence of *T. gondii* IgG, IgM and IgG+IgM antibodies were significantly higher ($p < 0.01$) in HIV patients with CD4 count <200 cells/ μ L compared with their counterparts with CD4 count ≥ 200 cells/ μ L irrespective of treatment status of the HIV patients (Table 2).

Among HAART-naïve HIV patients as well as those on HAART, the prevalence of IgM antibodies to *T. gondii* was significantly higher ($p=0.0268$ and $p=0.0243$, respectively) compared with the prevalence of IgG and IgG+IgM among those with CD4 count <200 cells/ μ L. While among HIV patients with CD4 count ≥ 200 cells/ μ L, the prevalence of IgG antibodies to *T. gondii* was significantly higher among HAART-naïve HIV patients ($p=0.0171$) and those on HAART ($p < 0.0001$) compared with IgM and IgG+IgM antibodies (Table 3).

Table 1: Prevalence of IgG and IgM antibodies to *T. gondii* among the study participants in Benin City, Nigeria

Antibody type	HAART-naive ^γ (n=264)	On HAART [†] (n=936)	Non-HIV [‡] (n=300)	Total [†] (n=1500)
Ig G	32(12.12)	127(13.57)	8(2.67)	167 (11.13)
IgM	46(17.69)	64(6.84)	3(1.00)	113 (7.53)
IgG+IgM	24(9.09)	23(2.46)	2(0.67)	49(3.27)
Total	102(38.64)	214 (22.86)	13(4.33)	329(21.93)

γ: p=0.0152; ‡: p=0.0890; †: p<0.0001

Table 2: Effect of CD4 count on the seroprevalence of *T. gondii* infection in Benin City, Nigeria

Antibody type	CD4 count (cells/μL)		P value
HAART-naïve	<200 (n=88)	≥200 (n=176)	
IgG	16 (18.18)	16 (9.09)	0.0532
IgM	31 (34.09)	15 (8.52)	<0.0001
IgG+IgM	20 (22.73)	4 (2.27)	<0.0001
On HAART	<200 (n=116)	≥200 (n=820)	
IgG	29 (25.00)	98 (11.95)	0.0002
IgM	34 (29.31)	31 (3.78)	<0.0001
IgG+IgM	17 (14.66)	6 (0.73)	<0.0001

Table 3: Effect of CD4 count on the prevalence of IgG and IgM antibodies *Toxoplasma gondii* in Benin City, Nigeria

Antibody type	No. positive (%)	P value
HAART-naïve		
CD4 count <200 cells/μL (n=88)		0.0268
IgG	16 (18.18)	
IgM	31 (35.23)	
IgG+IgM	20 (22.73)	
CD4 count ≥200 cells/μL (n=176)		0.0171
IgG	16 (9.09)	
IgM	15 (8.52)	
IgG+IgM	4 (2.27)	
On HAART		
CD4 count <200 cells/μL (n=116)		0.0243
IgG	29 (25.00)	
IgM	34 (29.31)	
IgG+IgM	17 (14.66)	
CD4 count ≥200 cells/μL (n=820)		<0.0001

IgG	98 (11.95)
IgM	31 (3.78)
IgG+IgM	6 (0.73)

DISCUSSION

Of the 1500 subjects recruited for this study, 329 (21.93%) had antibodies to *T. gondii*. The prevalence of *T. gondii* was significantly higher ($p < 0.0001$) among HAART-naïve HIV patients (38.64%) compared to those on HAART (22.86%) and among non-HIV subjects (4.33%). The finding that toxoplasmosis is higher among HAART-naïve HIV patients compared to those on HAART, agrees with a previous report (11). Toxoplasmosis is an opportunistic infection that is more prevalent among immunocompromised persons (9). HAART has been reported to improve the immunity of HIV patients (14). This may explain the lower prevalence of Toxoplasmosis among HIV patients on HAART and non-HIV subjects.

The presence of IgG anti-toxoplasma antibodies indicates past or chronic infection while the presence of IgM anti-toxoplasma antibodies indicates current infection (8, 15). The prevalence of IgG antibodies to *T. gondii* is higher generally among HIV patients on HAART and among non-HIV subjects, although it failed to reach statistical significance among the non-HIV subjects. This agrees with previous reports (16-18). However, the report of Daryani *et al.* (16) did not show any significant difference in the seroprevalence of anti-*Toxoplasma gondii* IgG and IgM among HIV and non-HIV subjects. Interestingly among HAART-naïve HIV subjects, the seroprevalence of current infection as indicated by presence of IgM antibodies (17.69%), was significantly higher compared to past infections. This observation is the first report from Benin

City, Nigeria and appears to be the first compared with other locations. Most studies detect IgG antibodies only to *T. gondii* and/or did not mention the treatment status of their HIV patients. The only study that mentioned treatment status did not compare the prevalence of IgG and IgM antibodies to *T. gondii*, although the prevalence of Toxoplasma IgG (46.2%) antibodies was higher than the prevalence of toxoplasma IgM antibodies (2.3%) (11). HAART-naïve HIV patients that were positive for *T. gondii* infection in this study, should be treated urgently and placed on HAART as *T. gondii* infection can result in life threatening encephalitis (2).

The finding that antibodies to *T. gondii* were significantly higher or associated with CD4 count < 200 cells/ μ L agrees with a previous report (8). This confirms *T. gondii* to be an opportunistic infection that can lead to death (19, 20). Both IgG and IgM anti-*T. gondii* antibodies were higher in HIV patients with CD4 count < 200 cells/ μ L. This agrees with the report of Mukherjee and Kumar (21) that IgG and IgM antibodies increases with decreasing CD4 count. Although HAART has been reported to improve the immunity of HIV patients (14), patients on HAART whose immunity may not have fully recovered are still susceptible to opportunistic infections, in this instance, *T. gondii* infection.

Among HIV patients with CD4 count < 200 cells/ μ L (irrespective of treatment status), the prevalence of anti-*T. gondii* IgM antibodies was significantly higher compared with IgG and IgG+IgM ($p < 0.05$). This indicates that immunosuppression among HIV patients predisposes them to acute *T. gondii* infection. On the other hand,

HIV patients with CD4 count ≥ 200 cells/ μ L had significantly higher prevalence of anti-*T. gondii* IgG antibodies. This indicates chronic infection. Reactivation of latent toxoplasmosis has been reported as the most common form of the infection in HIV/AIDS patients and is associated with toxoplasma encephalitis especially in patients with CD4 count < 200 cells/ μ L (10, 21).

In conclusion, anti-*T. gondii* IgM was more common among HAART-naïve HIV patients while anti-*T. gondii* IgG antibody was more prevalent among HIV patients on HAART. Although, *T. gondii* infection was more prevalent among HIV patients with CD4 count < 200 cells/ μ L, immunodeficiency was mostly associated with acute *T. gondii* infection.

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