Seroprevalence of Toxoplasma gondii IgG antibody in HIV-infected patients at the Lagos University Teaching Hospital

Background: Toxoplasmosis is caused by infection with a ubiquitous intracellular protozoan parasite, Toxoplasma gondii. With the advent of the HIV pandemic in Nigeria, toxoplasmic encephalitis has become one of the more frequent opportunistic infections and the most commonly implicated cause of focal brain lesions complicating the course of AIDS.

Objectives: This study was conducted to compare the pattern of seroprevalence of T. gondii (Toxo-IgG) antibodies among HIV-infected persons presenting with neurological complications and those without.

Materials and methods: Plasma specimens collected from 380 subjects were tested for Toxo-IgG antibodies by enzyme immunoassay technique and CD4 estimation by flow cytometry. Close-ended questionnaires were applied to all respondents to collect relevant data, with ethical approval from the hospital ethical committee. Plasma was obtained from two study groups comprising 300 HIV-positive respondents without neurological presentations, and 80 HIV-positive respondents with neurological complications.

Results: Seroprevalence of Toxo-IgG antibodies was 58% in the HIV-positive study group without neurological complications (of these, 79.2% were males and 38.5% were females) and 40% in the study group with neurological complications (46.2% of these were males and 28.6% were females). The overall seroprevalence of Toxo-IgG antibodies among the HIV-positive respondents (with and without neurological complications) was 54.2% (206 of 380). Seroprevalence of Toxo-IgG antibodies was lowest among the educated subjects (19% of the respondents with tertiary education) and among females in both study groups. A higher proportion of the subjects with neurological complications had CD4 cell count $<100$ cells/$\mu$L compared with respondents without neurological defects ($39\%$ vs $22.7\%$; $P=0.000$), but the seroprevalence of Toxo-IgG antibodies was higher in subjects without neurological complications ($45\%$ vs $31.3\%$; $P=0.000$).

Conclusion: Toxoplasmosis, though an important opportunistic infection in our environment, may not account for the majority of neurological complications observed in patients with HIV infection in our center.

Keywords: Toxoplasma gondii antibody (Toxo-IgG), seroprevalence, neurological complication

Introduction

Toxoplasmosis is caused by infection with a ubiquitous intracellular protozoan, Toxoplasma gondii. Reproduction is both sexual and asexual. Sexual reproduction occurs in definitive hosts mostly involving the cat family, and occasionally mice or rats. Asexual reproduction occurs in a wide range of animals, which includes man as intermediate host. The onset of cellular immunity against T. gondii is mediated by T-cells,
macrophages, and activities of type-1 cytokines (interleukin-1 and interferon gamma). This is accompanied by the transformation of the parasite into tissue cysts resulting in chronic infection.\(^3\) Humoral immune response is initiated by the production of specific Toxoplasma gondii antibodies toxoplasma antibodies (IgM and IgG).\(^3\) IgM antibody response occurs as an early event, and disappears within a few weeks or months, thus its presence in plasma indicates recent infection.\(^3,4\) IgG antibody production peaks within 1 to 2 months after infection but remains elevated for life.\(^4\) Several methods for diagnosis of complications including motor weaknesses and speech disturbances, seizures, cranial nerve abnormalities, sensory disturbances, cerebellar dysfunction, meningismus, movement disorders, and neuropsychiatric manifestations, and to determine the proportion of these patients whose neurological deficits may be remotely attributable to \(T. gondii\) infection

**Materials and methods**

**Study location**

This study was carried out at the Lagos University Teaching Hospital, Lagos State in the south-west region of Nigeria. Ethical approval for the study was obtained from the hospital ethical committee. Participants were recruited from the out-patient clinic of the hospital, which had over 6000 registered HIV-positive patients from different socioethnic backgrounds.

**Study population**

Three hundred and eighty subjects who screened and confirmed positive for HIV-1 or -2 were recruited by random sampling technique at the point of registration. The 380 HIV-positive respondents were separated into 2 study groups based on the presence of clinical evidence of neurological symptoms. Of the participants, 300 were without any obvious neurological symptoms while 80 of the HIV-positive participants presented with symptoms which included: neck stiffness, photophobia, tremors, irratational talk, paraesthesia, insomnia, and persistent headache, as isolated disorders or in any combination.

Patients with previous history of anti-retroviral drug therapy, cerebrovascular accidents, sepsin prophylaxis, pyrimethamine, and chemotherapy were excluded from the study.

**Sample collection and processing**

Two venous blood samples of 4.5 mL each were drawn from each subject into Na-EDTA specimen tubes. Plasma was obtained by centrifugation at 3000 rpm for 5 minutes, and stored at \(-20^\circ C\), prior to Toxo-IgG assay. All collected plasma were analyzed using ELISA by Immuno-comb Toxo-IgG kit manufactured by Orgencis Ltd, Yavne, Israel, with a sensitivity of 97.2% and specificity of 93.75%. CD4 estimation was carried out with a flow cytometer (Partec-model SL3).

**Interpretation of results**

Positive control must produce 2 spots on the card tooth to be valid; negative control must produce only an upper control spot to be valid. A lower spot with an intensity higher than or equal to that of positive indicates the presence of IgG antibody to \(T. gondii\).
Statistical analysis

The data were analyzed using statistical software SPSS version 11 (SPSS Inc, Chicago, IL). The data on qualitative variables were indicated by frequency and percentages, and quantitative variables by range and mean. Associations were verified by Chi-square with level of significance placed at $P < 0.05$ (5%).

Results

Demographic characteristics (Tables 1 and 2)

Mean ages of participants (HIV-positive subjects with neurological symptoms and HIV-positive subjects without neurological symptoms) were 39.5 ± 4.8 years and 38.4 ± 7.2 years respectively. A higher proportion of the subjects (140 of 380, 36.7%) were between the age range of 37 to 46 years.

Of the respondents, 196 were males (51.6%) and 184 were females (48.4%). Most respondents (264 of 380, 69.5%) were married. Only 31.6% of HIV-positive subjects had a tertiary education (120 of 380). The neurological symptoms manifested by these HIV-positive respondents were: headache (20%), neck stiffness (14%), photophobia (6.3%), paraesthesia (6.3%), tremor (20%), irrational talk (20%), and insomnia (14%). Of these, tremor, headache, and irrational talk constituted the majority of the clinical presentations (60%).

Seroprevalence of Toxo-IgG antibody among respondents (Tables 2 and 3)

Seroprevalence of Toxo-IgG antibody was 40% among the HIV-positive study group with neurological symptoms and 58% among the HIV-positive study group without neurological symptoms ($P = 0.00$).

There was a higher seroprevalence of Toxo-IgG antibodies among the age range of 37–46 years (102 of 140) representing 72.9% of the total in that age group. This was statistically significant at $P = 0.00$.

Toxo-IgG antibody and associated factors in HIV-infected respondents (Tables 3 and 4)

Of the 380 HIV-positive subjects, 206 were seropositive for Toxo-IgG antibodies (54.2%). Toxo-IgG antibody was more prevalent among the male subjects (70.4%). However this association was only statistically significant among the study group without neurological symptoms ($P = 0.00$).

CD4 count of all HIV-positive respondents ranged between 7 and 772 cells/µL, with a median count of 320 cells/µL. Of the HIV-positive study group without neurological complications who had CD4 count below 100 cells/µL, 79.4% were seropositive for Toxo-IgG antibody (statistically significant, $P = 0.00$), compared with 32.2% in subjects with neurological complications.

The association between Toxo-IgG antibody and HIV infection was more significant among the HIV-positive study group without neurological symptoms ($P = 0.00$).

Seroprevalence of anti-Toxoplasma gondii antibodies

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Toxo-IgG +ve N (%)</th>
<th>Toxo-IgG −ve N (%)</th>
<th>Total Chi²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>16–26</td>
<td>15 (50)</td>
<td>15 (50)</td>
<td>30</td>
<td>25.99</td>
</tr>
<tr>
<td>27–36</td>
<td>51 (50)</td>
<td>51 (50)</td>
<td>102</td>
<td>0.00</td>
</tr>
<tr>
<td>37–46</td>
<td>90 (75)</td>
<td>30 (25)</td>
<td>120</td>
<td>0.00</td>
</tr>
<tr>
<td>&gt;46</td>
<td>18 (38)</td>
<td>30 (62)</td>
<td>48</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Toxo-IgG +ve N (%)</th>
<th>Toxo-IgG −ve N (%)</th>
<th>Total Chi²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>114 (79)</td>
<td>30 (21)</td>
<td>144</td>
<td>0.00</td>
</tr>
<tr>
<td>Female</td>
<td>60 (39)</td>
<td>96 (61)</td>
<td>156</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HIV patients with neurological symptoms</th>
<th>HIV patients without neurological symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of subjects</td>
<td>80</td>
<td>300</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.6 ± 4.8</td>
<td>38.6 ± 7.2</td>
</tr>
<tr>
<td>Sex of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>52 (26.5)</td>
<td>144 (74.5)</td>
</tr>
<tr>
<td>Female</td>
<td>28 (15.2)</td>
<td>156 (84.8)</td>
</tr>
<tr>
<td>Educational background</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>16 (23.9)</td>
<td>51 (76.1)</td>
</tr>
<tr>
<td>Secondary</td>
<td>40 (20.7)</td>
<td>153 (79.3)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>24 (20)</td>
<td>96 (80)</td>
</tr>
</tbody>
</table>
Table 3: Association between anti-Toxoplasma gondii (Toxo-IgG) antibody and symptoms of neurological disorders in the HIV-positive subjects

<table>
<thead>
<tr>
<th>Toxo-IgG antibody status</th>
<th>Symptomatic subjects: N (%)</th>
<th>Asymptomatic subjects: N (%)</th>
<th>Total</th>
<th>Chi²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>32 (40)</td>
<td>174 (58)</td>
<td>206</td>
<td>8.24</td>
<td>0.00</td>
</tr>
<tr>
<td>Negative</td>
<td>48 (60)</td>
<td>126 (42)</td>
<td>174</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion
In this study, the data showed a Toxo-IgG antibody seroprevalence rate of 54.2% in HIV-infected persons. Earlier serological studies in many groups showed that the prevalence varied depending on the geographical location, between 15% and 68%. A recent US survey estimated a prevalence rate of 15% in the general population and 40% in people with HIV. Nissapatorn et al. evaluated the prevalence in 505 HIV-positive patients admitted to a hospital in Malaysia and reported a prevalence of 44.8%. Wanachiwanawin et al. estimated a prevalence rate of 53.7% in the HIV-infected population in Thailand. Meisheri et al. in India reported a prevalence rate of 30.9% and 67.8% in the immunocompetent group and HIV-infected persons respectively. Galva Ramirez et al. estimated a prevalence rate of 50% in the HIV-infected population in Mexico. In Africa, Lindstrom et al. evaluated 130 HIV-positive patients in Uganda and estimated a prevalence rate of 54%. The prevalence rate in this study equates well to that seen in the African study in Uganda, and that seen in Mexico.

The slight variations in prevalence rates obtained in this study compared with other earlier studies could be attributed to differences in geographical location. Infection is more common in warm climates, and at lower altitudes than in cold climates and mountainous regions. However, public enlightenment/health education, and high Human Development Index (HDI) standards could also be responsible for reduced infection.

The overall seroprevalence was highest (72.9%), in the 37 to 46 year age group, which is in agreement with an earlier report by Meisheri et al., which showed the highest prevalence within third and fourth decades of life. However, Nissapatorn et al. reported the highest prevalence rate within the second and third decades of life. The increasing prevalence rate with increasing age could be explained by increased risk of exposure to infection with increasing age. There is no statistical significant difference in age distribution between HIV-infected patients with neurological symptoms and those without.

Most Toxo-IgG antibody seropositive cases were males. The sex predilection could be attributed to the higher risk of consumption of meat (more likely to be contaminated) among Nigerian males. There was an association between a CD4 count <100 cells/µL and Toxo-IgG antibody seropositivity in HIV-positive subjects without neurological symptoms: 79.4% of the HIV-positive study group without neurological symptoms who had a CD4 count <100 cells/µL were seropositive for Toxo-IgG antibodies (P = 0.00). But this association was not observed in subjects with neurological symptoms (32.2%; P = 0.26). Studies by Eliaszewicz et al. in France, which included 172 HIV-infected subjects with diagnosed cerebral toxoplasmosis, showed that 79% of these patients had CD4 count <150 cells/µL. This result is contrary to findings in this study, which may further confirm the earlier position that neurological complications among HIV-positive subjects may be significantly unrelated to toxoplasmic infections. Johnson and Sayles studied resistance to T. gondii infection in mice lacking CD4 expression, and concluded that CD4-deficient mice exhibited impaired resistance to a challenge infection with virulent tachyzoites; therefore HIV infection increases susceptibility to T. gondii infection (not necessarily with toxoplasmic encephalitis). Thus CD4 serves as a reliable indicator for possible toxoplasmosis, and also contributes significantly to protection against chronic T. gondii infections via their role as helper cells for production of isotype-switched antibodies. However, findings from this study showed that only 40%...
of all respondents with neurological complications were seropositive for Toxo-IgG antibody compared with 58% of HIV-positive subjects without neurological complications. This implies that 60% of the subjects with neurological complications actually have other possible etiological factors; therefore the occurrence of neurological complications among participants in this study is not associated with higher incidence of Toxo-IgG antibody seropositivity compared with HIV-positive subjects without neurological deficit, thus implying that neurological complications observed in this study group might be from direct invasion of the neural cells by the HIV virus, or other common differentials.

Conclusion
In this study, the seroprevalence rate of Toxo-IgG antibody in HIV-infected patients, with and without neurological deficits, was evaluated. It may be concluded that though T. gondii infection is highly prevalent in our environment, it may not be responsible for the majority of cases of neurological disorders observed in our HIV-positive patients. However, extensive study in this area, using more accurate methodology including CT scan and PCR techniques, may be needed to establish the exact incidence of toxoplasmic encephalitis in these patients and evaluate differentials.

Disclosure
The authors declare no conflicts of interest.

References

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