Cryptococcus neoformans infection among human immunodeficiency virus patients on highly active antiretroviral therapy in Benin City, Nigeria

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ABSTRACT

Objective: Cryptococcus neoformans infection is a well-recognized AIDS defining illness among HIV patients. Against the background of no data on C. neoformans infection among HIV patients in Nigeria, we determined its prevalence among HIV patients on highly active antiretroviral therapy (HAART).

Methods: Blood samples were collected from 333 HIV patients on HAART (156 males; 177 females) and analysed for cryptococcal capsular antigen and CD4 count using standard techniques.

Results: Gender was not a significant risk factor for acquiring cryptococcal infection (OR = 1.40; 95%CI: 0.67-2.92, P = 0.471). The prevalence of cryptococcal infection was not significantly affected by age (P = 0.302). A CD4 count of <200 cell/μL had a significant risk factor for acquiring cryptococcal infection among HIV patients on HAART (OR = 4.06; 95%CI: 1.89-8.71, P = 0.0003).

Conclusion: An overall prevalence of 9.91% of C. neoformans infection among HIV patients on HAART was observed. HIV patients with a CD4 count of <200cell/μL had approximately a 2–9 fold increased risk of developing cryptococcal infection. The data presented will be useful in the epidemiology and management of C. neoformans infections among HIV patients on HAART.

Keyword: Cryptococcus neoformans, HIV, HAART, Nigeria.


INTRODUCTION

Infection with the pathogenic yeast Cryptococcus neoformans is a well-recognized complication of immunosuppression (1). C. neoformans meningitis occurs in 30% - 50% of human immunodeficiency virus (HIV) infected individuals in sub-Saharan Africa and the developing world (2). A recent analysis estimated that each year there are >900,000 cases of and 600,000 deaths due to cryptococcal meningitis globally, with most cases occurring in sub-Saharan Africa (2).

Cryptococcal meningitis is a common opportunistic infection and AIDS–defining illness in patients with late stage HIV infection, particularly in Southeast Asia and Southern and East Africa. Cryptococcal meningitis also occurs in patients with other forms of immunosuppression and in apparently immunocompetent individuals. In parts of sub-Saharan Africa with the highest HIV prevalence, cryptococcal meningitis is now the leading cause of community–acquired meningitis ahead of Streptococcus pneumoniae and Neisseria meningitis (3).

In HIV infected patients, cryptococcosis is associated with CD4 counts of <200 cell/μL (2,3). In the highly active antiretroviral therapy (HAART) era, the prevalence of C. neoformans infection had been reported to decrease (4). To our knowledge, there is no report on C. neoformans infection in our Institution and records of cerebrospinal fluid among non–HIV individuals in the last 5 years has not reported C. neoformans as being the cause of meningitis. Although HAART has been reported to decrease the prevalence of C. neoformans infections, it carries the additional burden of immune-reconstitution inflammatory syndrome in patients with C. neoformans infections (4). To our knowledge there are no reports of C. neoformans infections among HIV patients, especially those on HAART. Therefore, we determined the prevalence of C. neoformans infections among HIV patients on HAART, as well as the effect of age, gender and CD4 count on this prevalence.

MATERIALS AND METHODS

Study area

This study was carried out between 1st February 2010 to 31st January 2011, at the University of Benin Teaching Hospital. The hospital is a tertiary hospital with a referral status and centre for the United States President’s Emergency Plan for AIDS Relief (PEPFAR) and Institute of Human Virology, Nigeria (IHVN) HIV/AIDS intervention. The intervention involves free HIV screening, treatment and management of HIV patients.

Study population

A total of 333 HIV patients on HAART for 3 – 6 months were recruited for this study. The age range of the patients was 21 – 60 years and consists of 156 males and 177 females. The HAART regimen included zidovudine, nevirapine and stavudine. All patients had signs and symptoms of C. neoformans infections such as fever, headache, photophobia, phonophobia, cough, and altered mental status including personality changes. Informed consent was obtained from all patients prior to specimen collection. The Ethical Committee of the University of Benin Teaching Hospital approved the protocol for this study.

CD4 and cryptococcal capsular antigen testing

Ten ml of blood was aseptically collected from each patient and dispensed into ethylenediamine tetracetic acid (EDTA) and plain vacutainers. The EDTA sample was used to determine CD4 cell counts by flow cytometry (Partec, Germany) following the manufacturer’s instruction. Briefly, 20 μl of whole blood was placed in a Partec tube, and 20 μl of CD4+ T cell monoclonal antibodies was added. The mixture was then incubated in the dark for 15 minutes at room temperature after which 800μl of buffer was added. The tube was then placed in the flow cytometer for counting and the CD4+ T cells value obtained from a programmed computer connected to the instrument.
Sera obtained from the clotted samples in plain containers were used to detect cryptococcal capsular antigen using the latex-cryptococcus antigen detection system (Immuno-Mycologic, Inc, Norman, USA) following the manufacturer’s instructions.

Statistical analysis
The data were analyzed with Chi square ($\chi^2$) or Fisher’s exact test and odds ratio analysis using the statistical software, INSTAT® (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS
The prevalence of cryptococcal infection was higher in females (11.3%) than in males (8.33%). Although female gender appeared to be a risk factor for cryptococcal infection, this failed to reach statistical significance (Table 1). The prevalence of cryptococcal infection decreased with age from 14.04% in the 21 – 30 years age group to 6.90% in the 41 – 50 years age group, rising slightly to 7.02% in the 51 – 60 years age group. Age did not significantly affect the prevalence of cryptococcosis. Patients with CD4 cell counts of < 200 cell/µL had a significantly higher prevalence of cryptococcal infection than those with CD4 cell counts of ≥200 cell/µL, and CD4 counts of < 200 cell/µL was associated with cryptococcal infections among HIV patients on HAART (Table 1).

Table 1. Effect of gender, age and CD4 count on the prevalence of cryptococcosis among HIV patients on HAART

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. tested</th>
<th>No. positive (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>156</td>
<td>13 (8.3)</td>
<td>0.714</td>
<td>0.342,1.487</td>
<td>0.471</td>
</tr>
<tr>
<td>Female</td>
<td>177</td>
<td>20(11.3)</td>
<td>1.401</td>
<td>0.672,2.920</td>
<td>0.077</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>21 – 30</td>
<td>57</td>
<td>8 (14.0)</td>
<td>0.302</td>
<td></td>
<td>0.302</td>
</tr>
<tr>
<td>31 – 40</td>
<td>103</td>
<td>13 (12.6)</td>
<td></td>
<td></td>
<td>0.126</td>
</tr>
<tr>
<td>41 – 50</td>
<td>116</td>
<td>8  (6.9)</td>
<td></td>
<td></td>
<td>0.226</td>
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<tr>
<td>51 – 60</td>
<td>57</td>
<td>4 (7.0)</td>
<td></td>
<td></td>
<td>0.626</td>
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<tr>
<td><strong>CD4 count (cell/µL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 200</td>
<td>121</td>
<td>22 (18.2)</td>
<td>4.06</td>
<td>1.893,8.708</td>
<td>0.0003</td>
</tr>
<tr>
<td>≥ 200</td>
<td>212</td>
<td>11 (5.2)</td>
<td>0.246</td>
<td>0.115,0.528</td>
<td>0.162</td>
</tr>
</tbody>
</table>

OR = odds ratio; CI = confidence interval.

DISCUSSION
The incidence of infections caused by the encapsulated yeast C. neoformans has risen markedly over the past 20 years as a result of the HIV epidemic and increasing use of immunosuppressive therapies (3). Although incidence and mortality have decreased in the era of HAART, cryptococcal meningitis remains an important cause of morbidity and mortality in the AIDS population especially in the developing world (4).

An overall prevalence of 9.91% of cryptococcal infection among HIV patients on HAART was observed in this study. This is higher than 3.2% observed among HIV patients from sub-Saharan Africa (5). This is the first report of HIV–associated cryptococcosis in our Institution and perhaps in Nigeria. It has been reported that in countries with a large access to antiretroviral therapy, overall mortality and incidence of AIDS-defining opportunistic infections have been reduced dramatically (6), and indeed a 46% reduction in cryptococcosis in the HAART era was reported in France (7). The prevalence of 9.9% observed in this study is, however, lower than a prevalence of 30% in individuals of African origin in France (7). Although being of African origin has been reported to be associated with an increased risk of cryptococcosis in the HAART era (7), geographical location and time of analysis may play a more important role on the prevalence of cryptococcosis among HIV patients as the prevalence obtained in our study was lower than previously reported (2).

Age and gender did not significantly affect the prevalence of cryptococcal infection among HIV patients on HAART. Older age (35 – 45 years) was reported to be associated with cryptococcosis among HIV patients on HAART (7). This was not observed in our study. However, CD4 counts of < 200 cells/µL was significantly associated with cryptococcosis. This agrees with previous reports (1,2). It is important to note that those studies (1,2) did not state whether their patients were on HAART. HAART has been reported to improved CD4 counts (8). However, patients on HAART that still have low CD4 counts (< 200 cell/µL) are at an increased risk of developing opportunistic infections and in our study C. neoformans infections.

It has been reported that some of the antiretrovirals used in the HAART regime interact with antifungal agents used to treat cryptococcosis and HIV patients with cryptococcal meningitis who initiate antiretroviral therapy are at particularly high risk for the immuno-reconstitution inflammatory syndrome (IRIS) (4). Physicians should bear these in mind as they manage cryptococcal infections in HIV patients and indeed some have suggested that HAART should be initiated 2 – 6 weeks after commencement of antifungal therapy in an effort to reduce the occurrence of IRIS (4).

Our study underscores the importance of cryptococcal infections among HIV patients on HAART where an overall prevalence of 9.9% was observed and HIV patients on HAART with CD4 counts of < 200 cells/µL had a 2 to 9 fold increased risk of developing cryptococcal infections.
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REFERENCES

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