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EVALUATION OF PREDICTIVE CD4 COUNT AND CLINICAL RELIABILITY IN DETECTING CRYPTOCOCCUS NEOFORMANS AT KENYATTA NATIONAL HOSPITAL

Mdodo. R [3], Khayeka, C.W[1]. Songok, S.[4] and Choge, J.K.[2]

1. Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture and Technology, P.O Box 20617 Nairobi-00100, Kenya.

2. School of Health Sciences, University of Eastern Africa, Baraton. P.O Box 2500, Eldoret-30100, Kenya.

3 School of Public Health,C. D.C, University of Alabama at Birmingham, 1600 Clifton Rd Atlanta, GA30333, USA/1665 University Boulevard RPHB 217 Birmingham.

4. Chepkoilel University College, (A Constituent of Moi University), P.O. Box 1125-30100 Eldoret

Abstract

Cryptococcal meningitis (CM) caused by *Cryptococcus neoformans* is increasingly becoming a fatal fungal infection, especially among HIV/AIDS patients. This is because the HIV virus targets and destroys CD4 T cells (core cellular immune effector cells) thus causing immunosuppression. The objective of the study was to determine occurrence of CM, CD4T cells counts and assays, in patients either suspected or confirmed to have cryptococcal meningitis. The cross-sectional study design involved 51 HIV patients from KNH, during August-September 2008, sampled using Java and Applets method (2006). Results showed that CD4 T cells count among all the patients suspected to be infected with cryptococcal meningitis ranged from 0-937 cells/µL; majority had CD4 counts of ≤200 cells/µL. Since 43% of HIV patients with cryptococcal meningitis had CD4 counts of ≤143 cells/µL, a CD4 count of 143 cells/µL should be used as the lower limit below which HIV patients should be given prophylactic drugs for the disease, regardless of the prevailing clinical features seen in the affected patients.

Key words: CD4 T cell count; *Cryptococcus neoformans*; Cryptococcal meningitis; HIV

[1]Corresponding author E-Mail address: khayekachris@yahoo.com
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Introduction

Sub-Saharan Africa carries the highest burden of the acquired immune deficiency syndrome (AIDS) epidemic in the world with 25 million people living with the causative virus and in Kenya the prevalence of HIV among adults aged 15 – 49 years is estimated at 6.1% (UNAIDS, 2006; Waxman et al., 2008). Consequently the incidence of cryptococcal meningitis (CM) caused by *Cryptococcus neoformans*, which is a common complication of AIDS, has increased steadily to kill up to 60% of affected AIDS patients within a year (Mitchell and Perfect, 1995). Global estimates of the CM among AIDS patients are 6 – 8% in adults and 1% in children (Bii et al., 2007). According to UNAIDS (1998) CM prevalence is relatively higher in Thailand (25%) and Zaire (19%) than other countries.

The fungus *C. neoformans* is an environmental saprophyte that can infect the human brain or any body organ but it has a predilection for the lung and central nervous system (Bicanic and Harrison, 2005). The fungus can be diagnosed by direct microscopic examination of cerebrospinal fluid (CSF) using India ink, by culture and by serology (Mitchell and Perfect, 1995). Despite treatment the host response to *C. neoformans* through Immunological mechanisms are relatively well understood. There is general agreement supported by studies that a strong cellular immune response that produces granulomatous inflammation is essential for containment of this infection (Murphy, 1992; Levitz, 1992). This inflammation is primarily a Th-1 polarized response with requirement of Cytokines such as tumor necrosis factor, interferon gamma and 1L – 2 for recruitment of inflammatory cells (Huffnagle et al., 1991).It is also clear that core effector cells against *C. neoformans* include CD4 T cells and activated macrophages (Levitz et al., 1994; Hill, 1992). Cryptococcosis is initiated in the lung after inhalation of yeast cells of *C. neoformans* which are small (approximately 5.5μm in diameter) and minimally encapsulated. Yeasts that are not expelled by the respiratory epithelia may penetrate to the alveoli. In the alveolar spaces, the yeast cells are initially confronted by the alveolar macrophages (AMΦ). Whether active infection and disease follow this interaction depends largely on the competence of the cellular defenses of the host, as well as the number and virulence of the yeast cells. Cellular Immune mechanisms normally mediate a successful host response through activation of alveolar macrophages at the lungs for phagocytosis and in blood stream monocytes and monocyte-derived macrophages have demonstrated effective killing of the yeast cells by intracellular and extracellular mechanisms (Diamond et al., 1972).

Materials and Methods

Study area, Study Design, Inclusion and Exclusion Criteria

The cross-sectional study was conducted among HIV patients with symptoms of CM at Kenyatta National Hospital (KNH) in Nairobi, Kenya during the period between August and September 2009. KNH is the oldest and the largest referral and teaching hospital in Kenya and serves as the primary hospital for the 4 Million residents of the capital city, Nairobi. It was founded in 1901 as the native civil hospital. It has a staff capacity of 6,000 and 1,800 beds with an annual outpatient attendance of 600,000 visits and in-patient population of 89,000, 4000 of whom are HIV/AIDS patients receiving antiretroviral treatment (Gilly et al., 2000). The Study participants included HIV patients 13 years and older with suspected (physician's diagnosis based on clinical symptoms) cryptococcal meningitis (CM). CM was suspected in patients with (i) signs if meningeal irritation such as photophobia, neck rigidity, vomiting and headaches; (ii) Fever with altered mental state; (iii) Unexplained change in mental state, or headache. All patients suspected to have CM, who were willing to participate in the study and gave informed consent, were included. Patients who were previously seen in other hospitals and referred to KNH and those already admitted at KNH were also eligible for the study as long as they were suspected or confirmed to have CM. Children aged 13 and below, pregnant women and HIV negative patients were excluded from the study. The sample of 51 for the study was calculated using the java applets for power and sample size software (Lenth, 2006). Hence to meet the estimated numbers of

51 patients, 63 were screened, considering likely losses during follow-up. A structured questionnaire was also administered to each patient. It included questions on demographic characteristics, duration of illness, symptoms and signs on admission, Prior use of antifungal drugs, compliance with treatment and clinical status of HIV infection. Patient's records were also used to obtain information such as date of HIV diagnosis, CD4 counts, HIV viral load, previous illness and treatments.

CSF and whole blood collection for examination

Collection of cerebrospinal fluid (CSF) by lumbar puncture, processing and storage of specimens, confirmation for CM infections by the latex agglutination test, India ink and culturing in BBL and Sabouraud's Dextrose Agar (SDA) media were done. CD4 counts establishment from whole blood samples by tritest method using the BD flowcytometer and

HIV Viral load diagnosis through polymerase chain reaction (PCR) were also done as part of baseline data (from 3 months prior to admission to the time of admission for all patients without hospital records).

Results

A total of 51 samples were analyzed and 22 of them with CD4 count ≤200 cells/μL were found to have C. neoformans infections which translated to 43% occurrence in the cohort, yet
17 samples with similar CD4 levels that translated to 33% were negative for C. neoformans infection. The remaining 12 samples (equivalent to 24%) from patients with suspected clinical symptoms for CM and CD4 count >200 whose tests were done from a golden specimen (lumbar puncture) like the rest of samples turned out to be negative for *Cryptococcus neoformans* infection due to unaccounted reasons, but probability based on demographics was speculated (Table1).

Table 1: Results showing the CD4 counts of ≤200 and >200 respectively:

	% with CD4 count of ≤200cells/μL	% with CD4 count of >200cells/μL
Cryptococcus neoformans Positive	43%	0%
Cryptococcus neoformans Negative	33%	24%
Total	76%	24%

Table 1 shows the CD4 counts of the patients in the sample population. The box- and- whisker plot of the positive and negative tests against CD4 counts was used in order to evaluate the approximate CD4 counts that endanger the HIV/AIDS patients to contact *C. neoformans* infections.

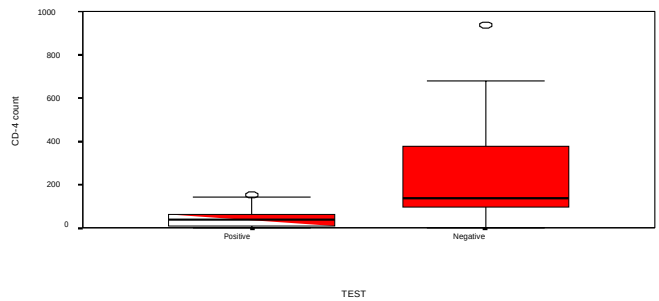

Figure 1: Box-and-whisker plot display for CD4 count among HIV patients who tested positive and negative for CM in Kenyatta hospital during the study period.

According to the box-and-whisker plots (Figure 1), there is no clear separation in the number of CD-4 counts between HIV patients who tested positive and negative for CM. However, the separation power of the plots is high, because only the whiskers overlap while interquartile ranges do not, indicating that the two groups are different. Unlike the Box-and-whisker plot which clearly shows presence of a difference between CD4 counts of *C.neoformans* positive and negative patients, the graph plot (Figure 2) indicates clear separation in the number of CD4 counts between HIV patients who tested positive and negative for CM with no patient who tested positive for CM exceeding a CD4 count level of 143cells/µL and a significant number of other patients lying within the same range with a variation of 0.4-0.5.

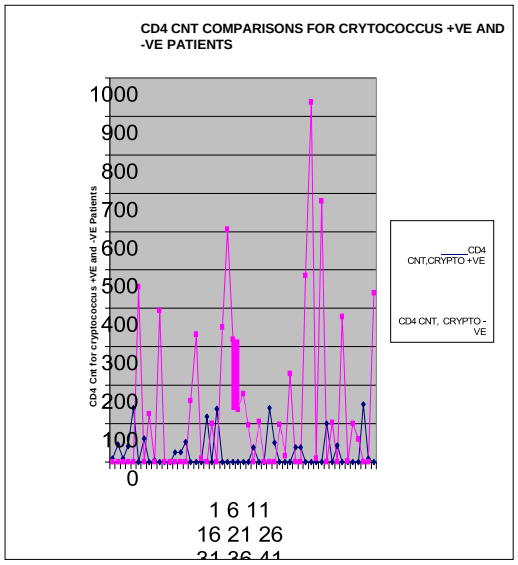

Figure 2: Graphical comparison of CD4 counts level for Cryptococcus +VE and -VE results of the inpatients under study.

Discussion

In the study it was clear that CM is common in KNH, occurring in 43% of HIV patients under the study presenting with headache, signs of meningeal irritation and fever with altered mental state. The high rate was not surprising, although the magnitude of the problem remained unclear to experts whtin KNH, despite a general observation of increased patients with the problem. Most reports on the severity of CM in Africa have originated from the Southern African region leading to the question whether or not the disease is less common in East and West Africa. The results gave a clue that CM is also an enormous burden in KNH as in

Zimbabwe (Hakim et al., 2000). However, headache and fever may also be commonly caused by malaria, bacterial meningitis, typhoid and brucellosis, among other diseases (Amexo et al., 2004; Barnish et al., 2004; Molyneux and Koram, 2007). However, it may be advocated that improvements in therapeutics is due to improvements in availability of diagnostics. During the time of the study, fluconazole was supplied by the Kenya Government, but this availability would have been useless without reliable *C. neoformans* detection kits and other related requirements. The value for improved tests can be confirmed by the 57% negative result for *C.neoformans* infection in patients who were potential suspects for CM and particularly those at CD4 counts of ≤200Cells/µL. India ink staining, which is the commonest method used on routine basis, exhibited disappointing sensitivity of 64%; hence increased availability of cryptococcal antigen tests that currently are rarely used during CM management in KNH and other Government hospitals, should be advocated. Although CD4 quantification is technically demanding and expensive, it is readily available in Kenya; hence should be more widely used for predicting CM diagnosis and its subsequent treatment, especially with coexisting headache and signs of meningeal irritation. The finding that low CD4 counts plus headache and signs of meningeal irritation was a reasonably sensitive and specific feature for CM that led to the detection of 43% positive CM results on testing is unfortunate. Firstly, it clearly requires CD4 quantification, which although is presently more available in Kenya, is also technically demanding and expensive.

Secondly, the finding of meningeal irritation and headache may provide high specificity but will inherently miss in earlier and better prognosis presentations as described in earlier research works. Hence the CD4counts of ≤143cells/µL plus headache and signs of meningeal irritation in patients with HIV should therefore prompt strong consideration of CM prophylactic treatment using fluconazole, since researches have shown that CM has high mortality in Africa (Hu et al., 2007). Alternatively periodic screening of such patients with serum cryptococcal antigen could be used, depending on the infrastructure and/or its availability. It was also observed that out of all patients who underwent lumbar puncture for whatever indication, 57% of them tested negative for *C. neoformans*; 33% out of the 57% had CD4 counts of <200cells/µL, thus showing that many HIV/AIDS patients with headache, signs of meningeal irritation and fever with altered mental state (AMS) do not necessarily have meningitis. In many resource-limited settings where lumbar puncture is not performed due to erratic availability of equipment, empirical use of antibiotics is the norm (Hu et al., 2007). However, data from the current study suggest that antibiotic use is often unnecessary; hence a waste of resources.

It was also observed that individuals with CD4 counts as low as 1-9 cells/ µL tested negative despite all clinical symptoms indicating possibility of CM. Further follow up showed that most of these individuals were on antiretroviral therapy. There were also individuals with CD4 counts as low as 0cells/µL who tested positive for *C.neoformans*. During follow up it was seen that majority of these individuals were initially on antiretroviral drugs (ARV's) but at some point they neglected the antiretroviral therapy; conversely, majority of those who turned out to be positive for *C. neoformans* infection had not initially been subjected to ARV's. This contrast can be a clue to research arguments trying to justify that ARV's utilization can delay or suppress immunosuppression levels for infection with opportunistic pathogens like *C. neoformans*.

Conclusions and Recommendations

Since majority of HIV patients (43%) with CD4 counts of ≤143 cells/µL had cryptococcal meningitis, a CD4 count of 143 cells/µL should be used as the lower limit below which HIV patients should be given prophylactic drugs for the disease, regardless of the prevailing clinical features seen in the affected patients.

Reasons to determine why not all patients with HIV had cryptococcal meningitis despite suggestive clinical features should be investigated.

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