Journal of Agriculture, Pure and Applied Science and Technology ISSN 2073-8749
Printed by Moi University Press

Evaluation of Predictive CD4 Count and Clinical Reliability in Detecting *Cryptococcus neoformans* at Kenyatta National Hospital, Kenya

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J. agric. pure appl. sci. technol. 5, 22-28 (2010); received November 20, 2009/April 17, 2010

Cryptococcus neoformans, which causes cryptococcal meningitis (CM), is increasingly becoming a fatal fungal infection, especially among HIV/AIDS patients, because the HIV virus targets and destroys CD4 T cells (core cellular immune effector cells), resulting in immunosuppression. The objective of the study was to determine occurrence of CM, CD4T cells counts and assays, in patients suspected/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 majority of HIV patients (43%) in the sample population, 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.

Key words: CD4 T cell count, *Cryptococcus neoformans*; *Cryptococcal meningitis* (CM) and HIV

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

This study was conducted 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 inpatients population of 89,000 patients, 4000 of whom are HIV/AIDS patients receiving antiretroviral treatment (Gilly, *et al.,* 2000).

Study Design, Inclusion and Exclusion Criteria

The cross-sectional study was conducted among HIV patients with symptoms of CM at Kenyatta National Hospital in Nairobi. 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, 2006-9). 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 which 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 *Cryptococcal 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**
Crypto Positive	43%	0%
Crypto 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. 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.

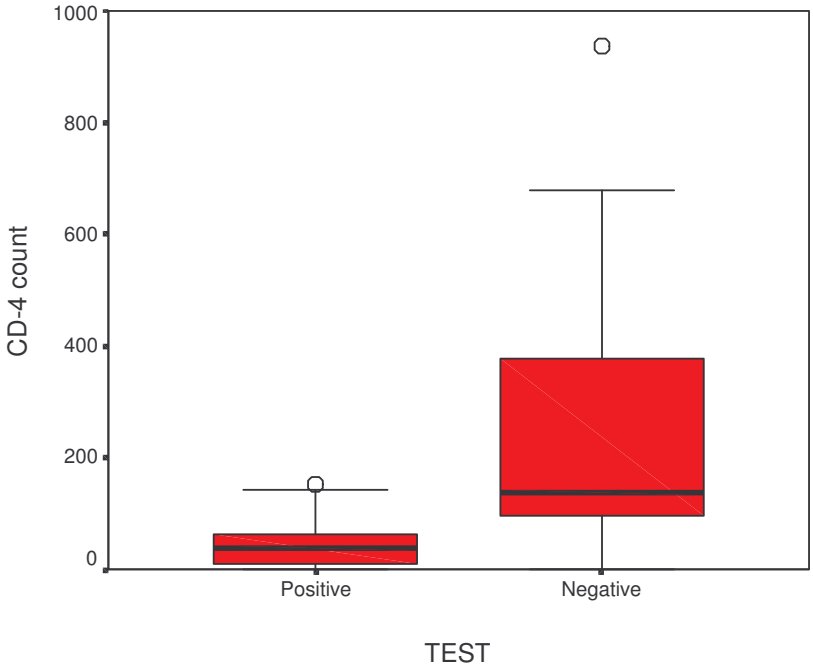

Figure 1: Box-and-whisker plot display for CD4 count among HIV patients who tested positive and negative for CM in Kenyatta hospital during the study period

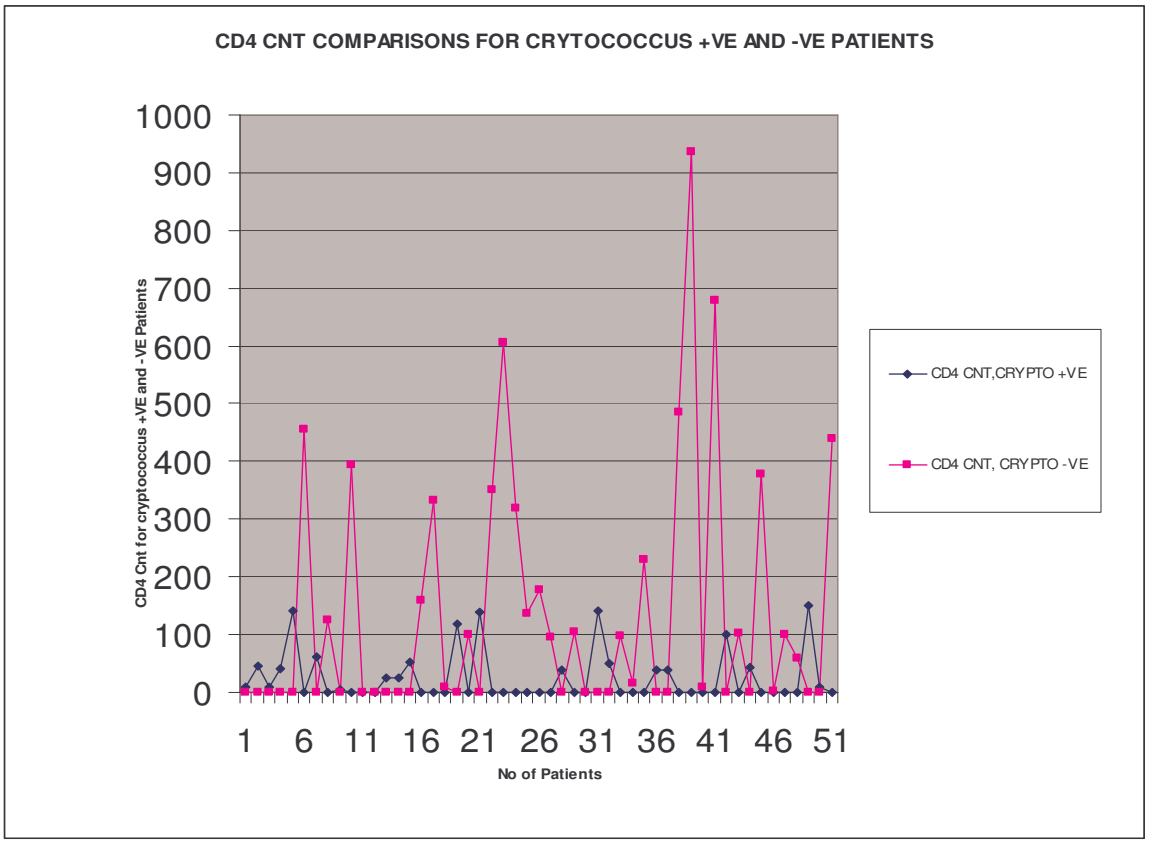

Figure 2: Graphical comparison of CD4 counts level for Cryptococcus +VE and -VE results of the inpatients under study.

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 clear despite a general observation of increased patients with the problem. Most reports on the severity of CM in Africa have originated from the South 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 the CM is also a 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, 2004; Barnish,et al., 2004; Molyneux and Koram, 2007). 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. Therefore, CD4counts≤143cells/µL plus headache and signs of meningeal irritation in patients with HIV should 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 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 been subjected to ARV's initially. 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.*

Conclusion

Since majority of HIV patients (43%) in the sample population, 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.

Acknowledgement

The authors sincerely thank the IREC of KNH for the approval of the collaborative research between CDC and Kenyatta National Hospital (KNH), which enabled the research to be carried out.

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