

Cryptococcal Antigenemia in HIV-Infected Patients with CD4⁺ T-cell Count \leq 200 cells/ μ l: A study from a tertiary care hospital

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ABSTRACT

Introduction: Cryptococcal meningitis is one of the leading opportunistic infections associated with high mortality. The present study was carried out to determine the prevalence of cryptococcal antigenemia in HIV-infected patients with CD4⁺ T-cell count \leq 200 cells/ μ l.

Material and methods: A cross-sectional study including a total of 100 blood samples of HIV-infected patients with CD4⁺ T-cell count \leq 200 cells/ μ l was carried out in a tertiary care hospital. The Cryptococcal Antigen Latex Agglutination Test was performed on serum separated from blood samples included in the study group. A positive cryptococcal antigenemia was diagnosed by positive latex agglutination test of cryptococcal polysaccharide antigen in serum. BMI of all patients included in the study group was calculated and WHO clinical staging of all patients was noted.

Results: Three cases out of 100 were positive for cryptococcal antigenemia. The positive cases showed correlation with low BMI and WHO Clinical stage II and III of HIV disease. In the present study, 33.33% and 66.67% of positive cases had CD4⁺ T-cell count within the range of 0-100cells/ μ l and 101-200cells/ μ l respectively.

Conclusion: It is important to implement routine screening for cryptococcal antigen among HIV-infected cases with CD4⁺ T-cell count \leq 200 cells/ μ l for early detection of cryptococcal antigenemia. It will help in identifying the risk of subsequent cryptococcal meningitis and initiation of pre-emptive antifungal treatment.

Keywords: Cryptococcal Antigenemia, CD4⁺ T-cell \leq 200 Cells/ μ l, HIV Positive

INTRODUCTION

HIV-infected patients develop a number of opportunistic infections due to severe immunosuppression. Cryptococcal meningitis is one such leading opportunistic infection with high mortality among the HIV positive patients.¹ Cryptococcal antigenemia is defined as having a positive serum cryptococcal antigen test.² The cryptococcal capsular polysaccharide antigen is detectable in peripheral blood before the symptoms of cryptococcal meningitis begin to show by an average of 22 days.³ This gives us an opportunity for early detection and treatment of subsequent cryptococcal meningitis, which will in turn, help to decrease the mortality. A study in India found 8% prevalence of cryptococcemia among adult AIDS patients with CD4⁺ T-cell count \leq 100 cells/ μ l.⁴ The occurrence of the cryptococcal antigenemia in patients with CD4⁺ T-cell count \leq 200 cells/ μ l in India is not well-known owing to very few studies carried out

in this matter. With this background, the present study particularly focuses on determining the prevalence of cryptococcal antigenemia among HIV-infected patients with CD4⁺ T-cell count \leq 200 cells/ μ l. The study will also be helpful to clinicians for identification of the risk of subsequent cryptococcal meningitis and for initiation of pre-emptive antifungal treatment in patients with cryptococcal antigenemia after its early detection. The World Health Organization (WHO) recommends screening of cryptococcal antigen in HIV positive patients with CD4⁺ T-cell count $<$ 100 cells/ μ l.⁵ An important aspect of our study is to guide the recommendations to include screening for the cryptococcal antigenemia at various threshold values of CD4⁺ T-cell counts within 200 cells/ μ l in HIV-infected patients, based on the findings of our study.

MATERIAL AND METHODS

A cross sectional study was carried out at a tertiary care hospital over a period of 6 months. The study was approved by the institutional ethics committee and the reference number of the approval is ND-ICMR 0217040-040. A written consent was requested from patients for collection of sample and inclusion in the project and confidentiality about their identity was maintained. A total of 100 blood samples collected from the HIV positive patients attending the ART OPD, with CD4⁺ T-cell count \leq 200 cells/ μ l were included in the present study. Patients of age group $>$ 18 years, those with confirmed diagnosis of cryptococcal meningitis or any other opportunistic infections and those on antifungal treatment

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were excluded from the study. The recent CD4⁺ T-cell count and WHO clinical staging of HIV/AIDS by presumptive clinical criteria of these patients was noted and the BMI was calculated.

1. Calculation of body mass index (BMI):

$$BMI = \frac{\text{Weight (in kgs)}}{[\text{Height (in m)}]^2}$$

2. Collection of blood and serum separation: Five ml of blood was collected aseptically from each patient included in the study. After allowing the blood sample to clot; it was centrifuged to separate the serum. The serum specimen was stored in refrigerator at 2-8°C till the test was performed.

3. Detection of cryptococcal antigen: The cryptococcal antigen was detected in the serum by Cryptococcal Antigen Latex Agglutination Test i.e. CALAS.

CALAS detection kit is a latex test that is capable of detecting the capsular polysaccharide of *Cryptococcus neoformans* in serum and CSF.

a. Preparation of serum specimen

- 200μl of serum specimen was added to 200μl of pronase solution.
- This was incubated at 56°C for 15 minutes and then boiled for 5 minutes.
- The solution was then allowed to cool at room temperature.

b. Testing of the serum specimen

- Negative controls were heat inactivated at 56°C for 30 minutes and positive controls were included.
- After cooling, about 25μl of antibody control and negative control was placed on appropriate rings.
- Then, about 25μl of serum specimen was placed on agglutination cards, in each of the two designated rings along with one drop of detection latex.
- Similarly, one drop of control latex was added into the rings.
- The card was then shaken to mix the contents together.
- The agglutination was read visually and the results were analyzed.

RESULTS

Present study reports 3% prevalence of cryptococcal antigenemia in HIV positive patients with CD4⁺ T-cell count ≤200 cells/μl (Figure-1). One patient out of three positive patients of cryptococcal antigenemia had a BMI value of 15.6 kg/m² which fell in the category of low BMI (<18.5 kg/m²) whereas the other two patients had BMI values of 19.53 kg/m² and 19.3 kg/m², respectively, which was in the normal BMI range of 18.5-24.9 kg/m² but towards the lower side (Figure 2). Two patients of cryptococcal antigenemia in the present study had WHO clinical staging of HIV/AIDS III, and one patient had WHO clinical staging of HIV/AIDS II

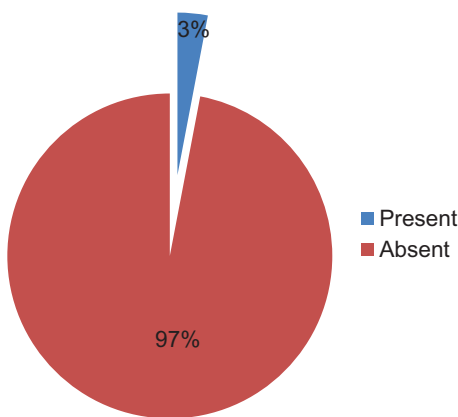


Figure-1: Prevalence of Cryptococcal Antigenemia (N=100)

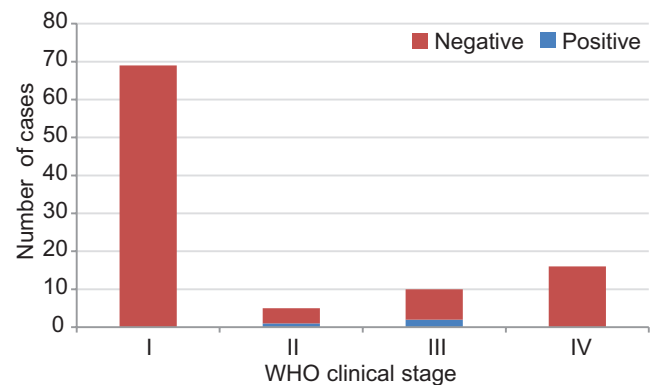


Figure-3: Correlation between Cryptococcal antigenemia and WHO clinical stages of HIV disease (N=100)

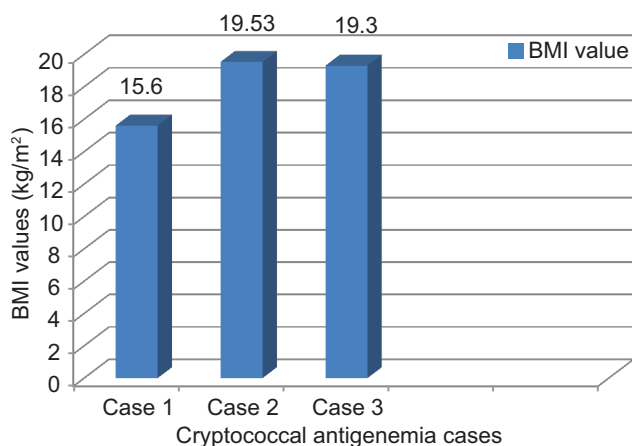


Figure-2: Correlation between cryptococcal antigenemia and BMI

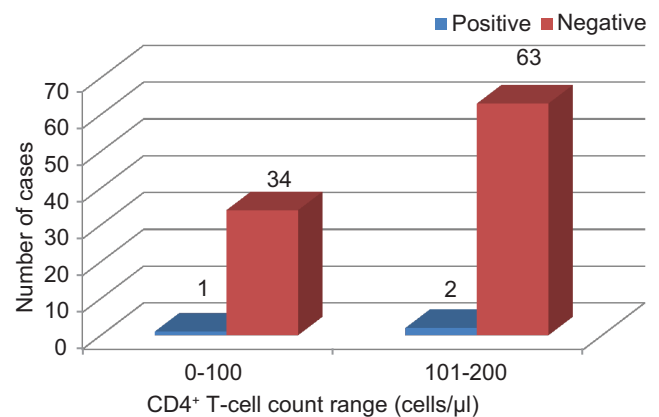


Figure-4: Correlation between Cryptococcal antigenemia and CD4⁺ T-cell count range (N=100)

(Figure 3). Two cases (66.67%) of cryptococcal antigenemia had their CD4⁺ T-cell count in the range of 101-200 cells/ μ l, whereas, one case (33.33%) had the CD4⁺ T-cell count in the range of 0-100 cells/ μ l (Figure 4).

DISCUSSION

The present study shows prevalence of cryptococcal antigenemia in 3% of patients with HIV-infection having CD4⁺ T-cell count \leq 200 cells/ μ l. Similar results were documented by Letang E et al⁶ and Pongsai P et al.⁷ Higher prevalence of cryptococcal antigenemia ranging from 6.2% to 19% were noted in studies by Kendi C et al⁸, Oyella J et al² and Kadam D et al.⁴ in HIV-infected patients with CD4⁺ T-cell count \leq 100 cells/ μ l. In the present study, HIV-infected patients with CD4⁺ T-cell count upto 200 cells/ μ l were included, whereas, in the studies reporting a higher prevalence, all the patients had CD4⁺ T-cell count of \leq 100 cells/ μ l. This could be a possible reason for the low prevalence of cryptococcal antigenemia in the present study as compared to the studies by Kendi C et al⁸, Oyella J et al² and Kadam D et al.⁴

In the present study, one positive case had a BMI of 15.6 kg/m² which was in the low BMI range (<18.5 kg/m²). Similarly, a study by Oyella J et al² in HIV-infected patients, found a low BMI of \leq 15.4 kg/m² to be one of the factors associated with cryptococcal antigenemia. Ganiem A et al⁹ also reported 70.4% of HIV-infected patients had a BMI of <18.5 kg/m². This correlation between low BMI and cryptococcal antigen positivity could be due to the dysfunctional immune system associated with low BMI and malnourishment which in turn increases the risk of cryptococcal antigenemia.²

The present study reports two cryptococcal antigenemia cases with BMI of 19.53 kg/m² and 19.30 kg/m², respectively, which inspite of being in the normal range of BMI (18.5-24.9 kg/m²) was on the lower side. Letang E et al⁶ found all the cryptococcal antigenemia cases to have a median BMI of 18.7 kg/m², which was comparable to the present study.

Interestingly, the present study found that two cases that tested positive for cryptococcal antigenemia had WHO Clinical stage III of HIV/AIDS and one case had WHO Clinical Stage II. A study had results in line with the present study with 1.3% of the cryptococcal antigenemia patients with WHO Clinical HIV/AIDS stage III.¹⁰ Another study¹¹ recorded 57.1% and 42.9% of the cryptococcal antigenemia patients had WHO Clinical stage I and II of HIV/AIDS, respectively. Contrary to the results of the present study, a study in Indonesia 81.1% of the cryptococcal antigenemia patients with WHO stage IV.⁹

An important finding of the study was the presence of cryptococcal antigenemia in both groups of patients with ranges of CD4⁺ T-cell count of 0-100 cells/ μ l and 101-200 cells/ μ l, with one out of three positive cases in the first group and two out of three positive cases in the second group, respectively. The WHO recommendation is screening for cryptococcal antigen in serum and pre-emptive antifungal therapy in those patients with a positive diagnostic test among HIV-infected patients with CD4⁺ T-cell count of <100 cells/

μ l in areas with high prevalence.⁵ Limiting the screening of cryptococcal antigenemia in HIV-infected cases to only patients with CD4⁺ T-cell count of <100 cells/ μ l would have led to missed diagnosis of two patients in present study. Thus, the findings of present study suggests reconsideration of guidelines to include higher screening threshold of CD4⁺ T-cell count of \leq 200 cells/ μ l in HIV positive patients.

This Institution based study has limitation like small sample size, which could have underestimated the results. Thus, a further large multi-centric study is needed covering larger population and for longer duration with screening of cryptococcal antigenemia in HIV positive cases.

CONCLUSION

Based on the findings of the present study, it is recommended to screen for cryptococcal antigen among HIV-infected patients with CD4⁺ T-cell count of \leq 200 cells/ μ l. The study also suggests that the clinicians should be more vigilant in screening for the cryptococcal antigenemia in HIV infected patients, especially those with low BMI and advanced WHO clinical stages of HIV disease.

Testing for early detection of cryptococcal antigenemia in HIV-infected patients with CD4⁺ T-cell count of \leq 200 cells/ μ l can serve as a potential screening tool to prevent risk of subsequent cryptococcal meningitis by initiation of pre-emptive antifungal treatment to lower the morbidity and mortality in these patients.

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