Asymptomatic Cryptococcal Antigenemia in People Living with HIV (PLHIV) with Severe Immunosuppression: Is Routine CrAg Screening Indicated in India?

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Abstract

Background: Cryptococcal meningitis (CM) is a common, life-threatening opportunistic infection (OI) among people living with HIV (PLHIV) in India. Serum Cryptococcal antigen (CrAg) positivity is predictive of future occurrence of CM and pre-emptive treatment reduces its mortality. Routine CrAg screening among PLHIV is not adopted by India’s national programme. This study evaluated the prevalence of CrAg and assessed CrAg positivity in predicting all-cause mortality among PLHIV.

Methods: This prospective study was conducted in a tertiary care, public health facility in New Delhi, India. Prevalence of CrAg was assessed in 128 ART naive adult PLHIV with CD4 < 100 cells/mm³ using a latex agglutination test. Age, gender, weight, body mass index (BMI), CD4 count, haemoglobin, serum albumin, and presence of other OI were evaluated as determinants of CrAg positivity. Subjects were followed up for occurrence of CM and mortality (all-cause) at 12 weeks and 6 months.

Results: The mean age of the subjects was 36.2±9.48 years, 73.4% were men, 21.09% women and 5.46% were transgender. The mean BMI was 18.4±2.53 kg/m² and 64% of subjects belonged to the lower socio-economic strata. Mean CD4 counts of the subjects was 54.9±26.58 cells/mm³ and 42.97% had CD4 < 50 cells/mm³. The prevalence of CrAg in the subjects was 3.125 % (4/128). None of the factors assessed showed statistically significant difference between the 2 groups, though CD4 count <50 cells/mm³, low serum albumin and presence of oral candidiasis had a stronger association with CrAg positivity. None of the subjects developed CM during follow up. At 12 weeks, 3/4 (75%) CrAg positive patients were alive compared to 118/124 (95.16%) of CrAg negative subjects. At 6 months, 50% (2/4) CrAg positive patients had died compared to 10.48% (13/124) CrAg negative (p<0.01)

Conclusions: Though CrAg prevalence in PLHIV with CD4<100 cells/mm³ is moderate, asymptomatic CrAg positivity among PLHIV with CD4 < 100cells/mm³ is significantly associated with higher all-cause mortality. CrAg testing is very cost effective and India’s National AIDS Control Programme should seriously consider routine screening among the severely immunosuppressed PLHIV.

Editorial Viewpoint

• Asymptomatic cryptococcal antigenemia is increasing in India and hence CrAg screening in asymptomatic PLHA is recommended.
• Treatment of these patients having high immunosuppression can prevent early death.

Introduction

Cryptococcal meningitis (CM) is an important, serious life-threatening Opportunistic Infection (OI) among PLHIV in India, especially among the severely immunosuppressed (CD4<100cells/mm³). The estimated global prevalence of CM is 1.9 to 4.57% among PLHIV and the worldwide annual burden of CM is estimated at 957,900 cases, resulting in an estimated 624,700 deaths within 3 months of cryptococcal infection.1 The highest prevalence of CM is seen in sub-Saharan Africa followed by Southeast Asia. The prevalence of CM in PLHIV is estimated at 2.79% in India.2 CM is associated with high mortality. In sub-Saharan Africa mortality rates of up to 70% have
been reported. High mortality rates of 55% in other low- and middle-income countries and nearly 20% in high income countries are estimated. Some of the reasons for these high mortality rates include delayed clinical suspicion and diagnosis due to subtle clinical manifestations, lack of access to lumbar puncture and cerebrospinal fluid (CSF) analysis, low sensitivities of diagnostic techniques used in these settings like India ink preparation, and non availability of Amphotericin B-based treatment regimen. Early and prompt diagnosis of CM and timely initiation of treatment are paramount in reducing the CM related mortality. CM presents as a sub acute to chronic meningitis and the diagnosis is often delayed in resource limited settings. To complicate matters, the clinical features of CM closely resemble tubercular meningitis, which is also endemic in these regions.

Hence it is important to screen for CM with a sensitive, specific, easily accessible and inexpensive test that will identify CM early. The detection of Cryptococcal antigen (CrAg) in serum is a simple test that is dramatically changing the screening protocols for CM among PLHIV. Serum CrAg has high sensitivity and specificity for the diagnosis of asymptomatic Cryptococcal infection among PLHIV. CrAg can be detected in serum or plasma weeks before the onset of the clinical features of CM and is estimated to precede the onset of CM symptoms by an average of 22 days and 11% of patients will have detectable CrAg >100 days prior to the onset of CM.

Routine screening for CrAg in PLHIV with advanced immunosuppression (CD4<100/mm^3) to detect asymptomatic Cryptococcal infection has been extensively studied in Africa. The reported prevalence of asymptomatic CrAg varies from 2% in Ghana, to 8.8% in Uganda and 13% in South Africa. In studies from Thailand and Cambodia, the prevalence of CrAg in PLHIV was 12.9% and 17.7% respectively in those with low CD4 counts. The WHO is also now recommending routine screening for CrAg in PLHIV with CD4 <100 /mm^3 in regions with prevalence of CrAg >3% and pre-emptive treatment with fluconazole. There is no published data on the prevalence of serum CrAg from India. The National AIDS Control Programme of India does not recommend screening for CrAg among asymptomatic PLHIV. This study, the first of its kind from India, assessed the prevalence and the determinants of CrAg among asymptomatic PLHIV. The institution is a designated Center of Excellence in HIV Care by the National AIDS Control Organization, Ministry of Health and Family Welfare, Govt. of India. The study was approved by the institutional Ethics Committee. ART naive PLHIV >18 years of age, with CD4 < 100/mm^3 who consented to participate in the study were assessed. All subjects with history of being treated for Cryptococcosis (definitive or presumptive) in the past or currently receiving fluconazole were excluded. All subjects were evaluated clinically by history and examination and WHO staging of HIV infection was done. Socio-demographic data was collected and baseline haematological and biochemical investigations as per NACO guidelines were done.

All subjects who fulfilled the study criteria were screened for CrAg detection in serum by Latex Agglutination (LAT) assay. CrAg detection was done using Meridian Bioscience, Inc., kits. Three to 4 ml of blood was taken and the clotted blood was centrifuged for 15 minutes. The serum separated was aspirated and 200 microlitres of serum was treated with 200 microlitres of pronase solution. This mixture was incubated at 56°C for 15 minutes. The mixture was then placed in a boiling water bath for 5 minutes. 25 microliters of this mixture was placed on a disposable reaction card. The card was then centrifuged for 5 minutes at 125 rpm and the results were read immediately. Those serum specimens with 2+ or greater reaction on a scale of 1-4 were considered to be positive for CrAg.

All subjects who were CrAg positive received antifungal treatment as per the 2011 WHO advisory.

The subjects were followed up for 6 months for outcome measures: whether they were alive or dead and whether they had developed an episode of CM in the intervening period. The first assessment was done at 12weeks and final outcome assessment was done at 6 months.

Statistical analysis: The mean, median, frequency distribution and the standard deviation were calculated using the subject characteristics. The prevalence of CrAg positivity was calculated. Among the PLHIV, comparison between the two groups (CrAg positive and CrAg negative) for quantitative data of determinants was done using unpaired t test and qualitative data of determinants was done using Fischer exact test to calculate the p value. Binary logistic regression was used to determine factors associated with positive serum CrAg. A p value <0.05 was considered statistically significant. The statistical analysis was done using SPSS software version 20.

Results

The study group comprised of 128 ART naive PLHIV. The mean age of the subjects was 36.2±9.48 years, 73.4% were men,
21.09% were women and 5.46% were transgender. Most patients (42.2%) were in the age group of 31 to 40 years. Among the subjects, 60.16% had either primary school education (37.5%) or were illiterate (22.66%) and 64.8% belonged to low socioeconomic status according to the modified Kuppuswamy classification. Heterosexual route of transmission was the most common route of HIV transmission in the subjects- 83.6%. Majority of the patients had fever as their presenting complaint at diagnosis of HIV infection (74 / 128) while 42 subjects were asymptomatic. Tuberculosis was the most common opportunistic infection (57 / 128) followed by oral candidiasis (17 / 128). Majority of the subjects belonged to WHO clinical stage 4 (n=48) followed by stage 3 (n=42). While 82% of the subjects had anaemia, 91% of the subjects had serum albumin <3.5g/dl. Ten subjects had Hepatitis B co-infection and 3 were co infected with Hepatitis C infection.

All subjects had CD4 < 100 cells/mm³. The mean CD4 counts of the study subjects was 54.9±26.58 cells/mm³ and 42.97% had CD4 < 50 cells/mm³. The prevalence of serum CrAg in the subjects was 3.125 % (4/128). Table 1 compares the clinical, demographic and laboratory data between CrAg positive and negative patients. There were no significant differences between CrAg positive and negative groups in terms of age, WHO clinical stage and Body weight/ BMI and CD4 count.

While assessing the determinants of CrAg positivity, none of the factors were statistically significant for association with serum CrAg positivity. However, age (p=0.26), CD4 count (p=0.105), and presence of oral candidiasis (p=0.284) had greater association with CrAg positivity than other determinants when assessed by binary logistic regression.

None of the subjects (CrAg positive or negative) developed CM during the follow up period of 6 months. At the follow up assessment at 12 weeks, 1 / 4 CrAg positive subjects died at home within the first week of diagnosis of HIV. Another CrAg positive subject had died due to disseminated tuberculosis at the end of 6 months of follow-up. The other 2 CrAg positive subjects who were started on both ART and prophylactic antifungal therapy were alive at 6 months. Among the CrAg negative subjects, 118/124 were alive at 12 weeks and 111 / 124 were alive at 6 months (Table 2).

### Discussion

Screening for serum CrAg has emerged as one of the most important tools to prevent Cryptococcal infections in PLHIV. The prevalence of CrAg among PLHIV is variable and depends upon the endemicity of Cryptococcal infections in the geographical region and also on the study population. It is recognised that 80% of CrAg positivity occurs among PLHIV with CD4<100 cells/mm³. Therefore routine screening for CrAg is recommended among PLHIV with CD4<100/mm³ as a priority among resource limited countries. In the WHO advisory released in 2011 for Cryptococcal disease, it has been conditionally recommended that universal screening for CrAg among PLHIV with CD4<100/mm³ should be adopted in all countries with a CrAg prevalence of >3%. In our study the prevalence of CrAg was documented to be 3.125%.

CrAg screening and treatment strategy has been shown to have a significant impact on reducing mortality among these patients. In a study by Meya et al it was found that CrAg-positive PLHIV without a history of CM, treated with fluconazole had higher odds of survival (OR: 34.6, 95% CI: 1.7 to 703) compared with those not treated with fluconazole. However, in a study by Meyer et al, there was no significant mortality benefit from CrAg screening demonstrated. In the present study also, CrAg positive patients had a significantly higher mortality both at 12weeks and 6 months (p<0.01). In a recent review of all studies on CrAg screening, it was concluded that that impact of “CrAg screen and treat strategy” on mortality overall was moderate. CrAg screening has also been demonstrated to be a highly cost effective strategy in the management of PLHIV. Meya et al demonstrated that the number needed to test and treat with CrAg screening to prevent 1 case of CM

### Table 1: Comparison of demographic, clinical and laboratory data among the CrAg positive and CrAg negative subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CrAg positive (n=4)</th>
<th>CrAg negative (n=124)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>43.5 ± 9.76</td>
<td>36.04 ± 9.38</td>
<td>0.26</td>
</tr>
<tr>
<td>Male gender</td>
<td>75%</td>
<td>73.44%</td>
<td>1.00</td>
</tr>
<tr>
<td>BMI in kg/m²</td>
<td>18.2 ± 0.83</td>
<td>18.4 ± 2.57</td>
<td>0.79</td>
</tr>
<tr>
<td>WHO clinical stage 3</td>
<td>50%</td>
<td>32.25%</td>
<td>0.597</td>
</tr>
<tr>
<td>WHO clinical stage 4</td>
<td>25%</td>
<td>37.9%</td>
<td>1.00</td>
</tr>
<tr>
<td>CD4 cells/mm³</td>
<td>35.25 ± 19</td>
<td>55.53 ± 26.52</td>
<td>0.105</td>
</tr>
<tr>
<td>Other co-existing OI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>50%</td>
<td>47.43%</td>
<td>0.99</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>25%</td>
<td>13%</td>
<td>0.284</td>
</tr>
</tbody>
</table>

The prevalence of serum CrAg in the subjects was 3.125 % (4/128).
was 11.3 persons and the cost per disability-adjusted life year (DALY) saved was $21. Jarvis et al\textsuperscript{13} in a study from South Africa showed that CrAg screening followed by treatment with fluconazole cost USD 51 per person per year compared to USD 207 per person per year for no screening at all. The most significant finding was that this impact was seen even in situations with prevalence of CrAg antigenemia as low as 0.6%. Though our study did not do a formal cost effectiveness analysis, it is estimated that the approximate cost of performing 1 CrAg test is $4, compared to $2 400 for the in-hospital care of 1 patient with CM. This is the scenario when the test used for CrAg determination in this present study was latex agglutination (LAT).

The CrAg screening strategy has been boosted by the development and approval of the newer Lateral flow assay (LFA) technique. LFA is a rapid diagnostic test that has emerged as an ideal point of care test for detection of CrAg.\textsuperscript{14} The test is simple to perform, does not require any specialised equipment or techniques or any training of lab personnel. Only 1 drop of body fluid (serum, plasma, CSF or urine) is required for the test which does not require any refrigeration also. Most significantly LFA costs only approximately 1-2 USD per test which is affordable in the resource limited settings. LFA has demonstrated high sensitivity and specificity for CrAg.\textsuperscript{14}

**Conclusions**

The present study is the first published study from India on CrAg detection in asymptomatic PLHIV. There is no published data from India on the prevalence of CrAg among PLHIV. This study has important implications for the National AIDS Control programme. Though this is a single center study, it still highlights the increased risk of mortality among PLHIV with CrAg positivity. A larger, multicentre study using LFA based CrAg screening will provide more data. The programme must consider implementation of universal, routine CrAg screening of PLHIV with CD4 <100/mm\textsuperscript{3} followed by pre-emptive fluconazole treatment for CrAg positives to prevent CM and its associated mortality.

**References**


