International Journal of Advances in Medicine
Kolligouda MG et al. Int J Adv Med. 2025 Sep;12(5):473-477
http://www.ijmedicine.com
pISSN 2349-3925 | eISSN 2349-3933
DOI: https://dx.doi.org/10.18203/2349-3933.ijam20252531

Original Research Article

The prevalence of asymptomatic Cryptococcal antigenemia in people living with human immunodeficiency virus with severe immunosuppression

Manjunath G. Kolligouda[1]*, Rajeev L. Khare[1], Prachi Dubey[1], Nikita Sherwani[2], Devpriya Lakra[1]

[1]Department of Medicine, Pt. JNM Medical College, Raipur, Chhattisgarh, India
[2]Department of Microbiology, Pt. JNM Medical College, Raipur, Chhattisgarh, India

Received: 21 July 2025
Revised: 13 August 2025
Accepted: 14 August 2025

***Correspondence:**
Dr. Manjunath G. Kolligouda,
E-mail: kolligouda@gmail.com

ABSTRACT

Background: Cryptococcal meningitis is a life-threatening disease among human immunodeficiency virus (HIV) patients specially with severe immunosuppression. Cryptococcal antigen (CrAg) can be detected well before the development of disease as CrAg circulates before the progressing to meningitis so if serum CrAg positive in asymptomatic patients the patients may develop cryptococcal meningitis in future so treatment in asymptomatic patients who are positive for CrAg can reduce the mortality.
Methods: This was cross-sectional study in which CrAg was tested among 84 PLHIV patient with CD4 count of less than 200/mm^3 using latex agglutination test. Age, gender, World Health Organization (WHO) staging, ART regimen, haemoglobin level and presence of other opportunistic infection were added as determinants of CrAg positivity.
Results: Mean age among the study subjects was 39.19 years. 72.6% were males and 27.4% were females. 77.4% belong stage 1 of WHO,6% to stage 2,15.4% to stage 3, and 1.2% to stage 4. Mean CD4 count of subjects was 94.70 cells/mm^3. 54.7% of the subjects had CD4 count of less than 100cells/mm^3 and 45.3% subjects had CD4 count of more than 100 cells/mm^3 subjects were tested positive for CrAg with prevalence being 6%. Mean CD4 count in subjects who tested positive was 34.20 cells/mm3 and in subjects who tested negative was 98.53 cells/mm^3.
Conclusions: Prevalence of asymptomatic cryptococcal antigenemia was found to be 6% in PLHIV with CD4 count less than 200 cells/mm^3 and prevalence was about 10.8% in subjects with CD4 count of less than 100 cells/mm^3 compared to 0% in subjects with CD4 count of 100-200 cells/mm^3. As the mortality of cryptococcal meningitis is very high and testing CrAg is cost effective if done in large scale.

Keywords: Cryptococcal meningitis, High mortality, Low CD4 count, Cryptococcal antigen, Asymptomatic patients, Routine screening

INTRODUCTION

Cryptococcal meningitis is a serious and life-threatening opportunistic infection in people living with HIV with very high mortality rate. The global burden of cryptococcus was recently estimated to be around >1 million cases with >6,00,000 deaths annually especially within 3 months of infection.[1] Global prevalence is estimated to be around 1.9-4.57% in PLHIV. The prevalence of cryptococcal meningitis in India among PLHIV is estimated to be 2.79%.[2] Estimated global prevalence of cryptococcal meningitis to be 1.9 to 4.57% among PLHIV.[3]

Sub Saharan Africa and south east Asia dominate in terms of case distribution as well as the mortality. In some sub-Saharan countries mortality is nearly 70%. The main

reason for this high mortality is delayed suspicion of the disease. For the diagnosis of the disease, the need of lumbar puncture and India ink preparation all gets delayed with late suspicion and also the clinical manifestations are very subtle which adds up to more difficulty in diagnosis. And the non-availability of Amphotericin based treatment regimen especially in many parts of underdeveloped countries leads to increased mortality. So, the screening or early detection of the disease is paramount importance in detection of disease and reducing the mortality.

Keeping in mind the above-mentioned difficulties of diagnosis, screening of disease becomes an important thing. The test to be used for screening should be simple, sensitive, specific, easily accessible and cost effective. Cryptococcal antigen (CrAg) serves that purpose. Detection of CrAg is simple and can be done with different principles namely enzyme immunoassay, latex agglutination assay and most recent one lateral flow assay. CrAg detection with latter two methods have high specificity and sensitivity. CrAg is detectable at least about 3 weeks (median) before the onset of symptoms of CM thus its detection and treatment of patients positive for the same is an important area which could lead to reduction in mortality of PLHIV.[4] Existing prevalence data for CrAg antigenemia are mostly from resource-limited settings and range from as low as 2% in northern Vietnam to 21% in Benin City, Nigeria on the basis of this data WHO in 2011 recommended that countries with a prevalence of CrAg of more than 3% in their population should consider routine screening and treatment for cryptococcal antigenemia even before ART initiation for ART-naïve adults with a CD4 T-cell count <100 cells/µl. This recommendation currently is followed only in South Africa, Rwanda and Mozambique. With regards to the cost effectiveness of the antigen detection, a study from Uganda concluded that the benefits of screening exceeded the costs.[5]

As mentioned above, WHO recommends for routine screening for cryptococcus antigen in PLHIV patients in countries with prevalence of cryptococcal meningitis more than 3% with CD4 count less than 100 cells/mm^3.

So, our objective for the study is to identify individuals with asymptomatic cryptococcal antigenemia in people living with HIV with severe immunosuppression by detecting CrAg in their serum.

METHODS

This was a cross-sectional study conducted in Department of Medicine and ART centre in Pt. Jawaharlal Nehru Memorial Medical College and associated Dr. Bhimrao Ambedkar Memorial Hospital, Raipur, Chhattisgarh, in the period between January 2019 to August 2020. The patient selected were registered in ART centre with CD4 count less than 200 cells/mm^3 and more than 18 years were registered for the study and assessed and patients who had full-fledged meningitis and who were on fluconazole or took fluconazole within 15 days were excluded. All the participants were clinically evaluated and examined and WHO staging was done and hepatitis B status was checked baseline investigations were done.

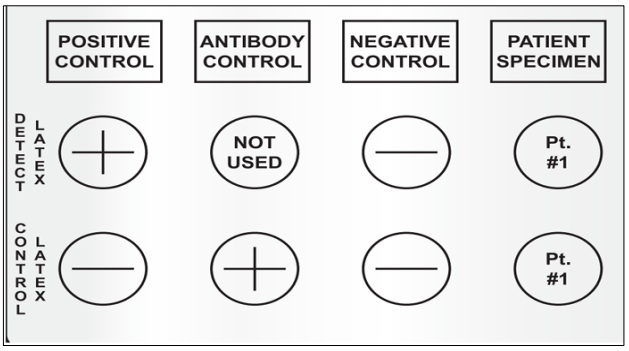

Figure 1: Positive control: this well contains a known positive sample that should react with the test reagents, ensuring the reagents are functional and the test is working correctly; antibody control: this control helps to confirm that the latex particles themselves are not causing non-specific agglutination and that any positive reaction observed is due to the presence of the target antigen or antibody; negative control: this well contains a known negative sample or diluent that should not react with the test reagents; patient specimen: this well contains the patient's sample (e.g., serum, csf) being tested for the presence of specific antigens (like cryptococcal antigen) or antibodies. the result in this well is compared to the controls to determine if the patient is positive or negative for the target substance.

Patients who satisfied the above criteria were tested for CrAg using latex agglutination assay (LFA has demonstrated high sensitivity and specificity for CrAg), which was done using cryptococcal antigen kit.[6] For that, 4 ml of patient's venous whole blood (detection of CrAg in serum or CSF has shown to have predictive value for future cryptococcal meningitis thus it has an important significance in management of PLHIV), was taken and the clotted blood was centrifuged for 15 minutes, then serum is aspirated in a sterile container and sealed and was processed immediately, refrigerated, preserved by freezing at -20^{0}C or by adding thimerosal to provide a final concentration of 0.01%.[7] 200 micro litres of serum is mixed with 200 micro litres of Pronase solution. Then incubated at 56 degrees for 15 minutes and then cooled, then solution placed in boiling water for 5 minutes to terminate enzymatic digestion and cooled to room temperature and used for the test (for tittering purposes, patient specimen was diluted 1:2 with the Pronase solution). Negative control was heat inactivated at 56 C for 30 minutes. It was heat inactivated each day of use (controls do not need to be run on each card with each patient sample).

Reaction cards are taken 25 µl of the antibody control and negative control placed into the appropriate rings and 25 µl of patient specimen placed in each of the two designated

rings and centrifuged at 125 rpm and results read and labelled from 1-4 and one with 2+ taken as positive. Negative (-) = a homogeneous suspension of particles with no visible clumping. One plus (1+) = fine granulation against a milky background. Two plus (2+) = small but definite clumps against a slightly cloudy background. Three plus (3+) = large and small clumps against a clear background. Four plus (4+) = large clumps against a very clear background.

After culminating the results statistical analysis was done. Mean, mode, median, standard deviation, frequency and distribution were calculated. CD4 was compared with age, sex and CrAg. CrAg test result was compared with different CD4 count groups. Prevalence of CrAg positivity was calculated. Fischer test was used to calculate p value and p value of less than 0.05 is considered as statistically significant. Statistics found using statistical package for the social sciences (SPSS) v20 software.

RESULTS

Among 84 HIV patients tested in our study, 72% were males and 28% were females and mean age among study subjects was 39.19 years. Maximum study subjects were in the age group of 31-40 years. 47% of study subjects didn't have any symptoms, and among patients who had symptoms most common was generalized weakness in 20% of the individuals. Considering opportunistic infections, 87% of subjects didn't have any opportunistic infection and among the subjects who had opportunistic infection, pulmonary tuberculosis was most common in 10.7% of subjects. 77.4% of subjects belong WHO HIV stage 1 and 6% to stage 2, 15.4% to stage 3 and 1.2% to stage 4. 2 subjects were co-infected with hepatitis B.

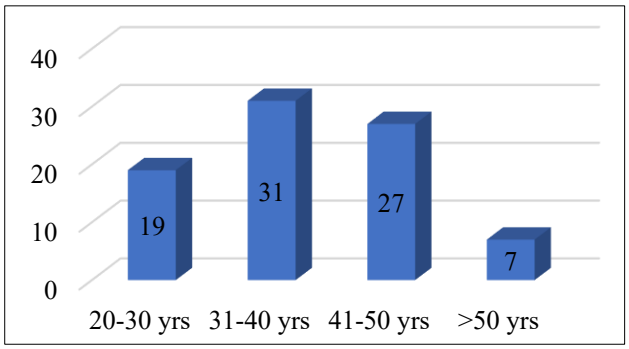

Figure 2: Demographic data.

Table 1: Comparison of test with CD4 count.

Variables	Cryptococcal antigen test		Prevalence in respective CD4 group (%)
	Positive	Negative	
Patients with CD4 count <100 cells/mm^3	5	41	10.8
Patients with CD4 count >100 cells/mm^3	0	38	0

Table 2: Statistics related to age, ART duration and CD4 count.

Cryptococcal antigen test	N	Mean	Standard deviation	Standard error mean	P value
Age					
Negative	79	38.89	9.338	1.051	0.520
Positive	5	43.00	13.096	5.857	
ART duration					
Negative	79	721.97	989.138	111.287	0.107
Positive	5	619.00	1085.267	485.346	
CD4 count					
Negative	79	98.53	53.038	5.967	0.010
Positive	5	34.20	34.142	15.269	

The prevalence of cryptococcal antigenemia in our study was found to be 6% in CD4 count less than 200 cells/mm^3. Table 1 shows subjects who were positive for CrAg had CD4 count of less than 100 and none of the subjects were positive with CD4 count between 100-200 cells/mm^3 and prevalence of cryptococcal antigenemia among subjects with CD4 count less than 100 cells/mm^3 was 10.8%.

Table 2 compares age with CrAg test, mean age among subjects with CrAg test positive was 43.00 years and mean age among subjects with CrAg test negative was 38.89 years, with p value being 0.520 shows there is no statistically significant correlation. Similarly, mean ART duration among CrAg positive subjects was 619.00 days, and among CrAg negative subjects was 721.97 days with p value being 0.107, statistically not significant.

CD4 count among subjects who tested positive for CrAg had mean CD4 count of 34.20 cells/mm^3 and among who tested negative had mean CD4 count of 98.53 cells/mm^3. P value was 0.010 which is statistically significant and showing correlation between low CD4 count with CrAg positivity.

DISCUSSION

Screening of CrAg is an emerging topic especially among the countries where it is endemic in PLHIV. As the prevalence varies geographically, some countries adopted screening in their national programs. It is recognised that 80% of CrAg positivity occurs among PLHIV with CD4. In 2011 WHO mentioned that in countries where the prevalence of cryptococcal antigenemia is more than 3% among PLHIV with CD4 count less than 100 cells/mm^3. So routine screening is indicated among these countries. Detection of CrAg in serum or CSF has shown to have predictive value for future cryptococcal meningitis thus it has an important significance in management of PLHIV.[9] CrAg is detectable at least about 3 weeks (median) before the onset of symptoms of CM thus its detection and treatment of patients positive for the same is an important area which could lead to reduction in mortality of PLHIV.[10] If we consider the cost, CrAg detection is cost effective if done in a large scale.[11] In study done in Delhi, India found asymptomatic cryptococcal antigenemia in PLHIV in severe immunosuppression patients with CD4 count less than 100, prevalence of positivity found to be 3.125%.

Kumar et al analysed 40 diagnosed patients of cryptococcal meningitis, and found mean age of these patients was to be 40.0±9.1 years.[12] And most patients were in the age group of 25-49 years.

Meya et al observed in his cohort study that 50 persons (8.2%) were serum CrAg positive when starting ART of 295 people with CD4+ ≤100 cells/µl.[13] They demonstrated the cost-effectiveness of CrAg testing in resource-limited settings in persons with CD4+ ≤100 cells/µl initiating ART and that ART alone is insufficient. We believe serum cryptococcal antigen screening should be integrated in national ART treatment programs in Sub-Saharan Africa, specifically targeting patients with severe immunosuppression (CD4+ ≤100 cells/µl), as CrAg screening is both cost-effective and affordable to reduce early mortality on ART. Ford et al stated that in a previous meta-analysis established a pooled CrAg positivity prevalence of 2.0% for PLHIV with CD4 100–200 cells/mm^3.[14]

Within one and half year of stipulated time of the study and with ongoing crisis throughout the world (COVID 19), only 84 patients were able participate in the study, so it was a small-scale study. So, actual prevalence may vary. Even though studies related to CrAg has been conducted vastly in African countries, studies in India are very limited so comparison of various parameters couldn't be done.

CONCLUSION

In our study we found that the prevalence of asymptomatic cryptococcal antigenemia in people living with HIV with severe immunosuppression was found to be 6% with CD4 count <200 cells/mm^3 and 10.86% with CD4 count less than 100 cells/mm^3. WHO advisory for cryptococcal disease in 2011 stated, it has been conditionally recommended that universal screening for CrAg among people living with HIV with CD4 count <100 cells/mm^3 should be adopted in all countries with cryptococcal antigen prevalence of >3%. So many studies have been conducted and shown that treatment of the patient who were found positive for CrAg before the development of the meningitis has reduced the mortality compared to the patient who were not treated. CrAg can be detected 3 weeks before the onset of the symptoms so it's a beneficial to have a mandatory screening of CrAg as advised by WHO when the prevalence is more than 3%. NACO doesn't include the guideline for routine screening for the CrAg in India. So, this study and many more studies come can determine the prevalence of asymptomatic CrAg prevalence among PLHIV with severe immune-suppression in India so that NACO can consider screening to be included in their guidelines because CrAg testing is cost effective, with specificity and sensitivity over 99%. The WHO is also now recommending routine screening for CrAg in PLHIV with CD4 3% and pre-emptive treatment with fluconazole.

ACKNOWLEDGEMENTS

The authors would like to thank all teachers of Department of Medicine of Dr. BRAM Hospital Raipur. They also thank Department of Microbiology for helping in conducting the test.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. 2009;23:525-30.
2. Baradkar V, Mathur M, De A, Kumar S, Rathi M. Prevalence and clinical presentation of Cryptococcal meningitis among HIV seropositive patients. Indian J Sex Transm Dis. 2009;30:19-22.
3. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. 2009;23(4):525-30.
4. French N, Gray K, Watera C, Nakiyingi J, Lugada E, Moore M, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. AIDS. 2002;16(7):1031-8.
5. Kato M, Long NH, Duong BD, Nhan do T, Nguyen TT, Hai NH, et al. Enhancing the benefits of

antiretroviral therapy in Vietnam: towards ending AIDS. Curr HIV/AIDS Rep. 2014;11:487-95.
6. Rajasingham R, Meya DB, Boulware DR. Integrating Cryptococcal Antigen Screening and Pre-emptive Treatment into Routine HIV care. J Acquir Immune Defic Syndr. 2012;59:85-9.
7. Meya DB, Manabe YC, Castelnuovo B, Cook BA, Elbireer AM, Kambugu A, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < 100cells/ml to start HIV therapy in resource limited setting. Clin Infect Dis. 2010;51:448-55.
8. Jackson AT, van der Horst CM. Editorial Commentary: Cryptococcosis in AIDS: New Data but Questions Remain. Clin Infect Dis. 2016;62:588-9.
9. McKenney J, Smith RM, Chiller TM, Detels R, French A, Margolick J, et al; Centers for Disease Control and Prevention.: Prevalence and correlates of cryptococcal antigen positivity among AIDS patients--United States, 1986-2012. MMWR Morb Mortal Wkly Rep. 2014;63:585-7.
10. Anuradha S, Narayana AH, Dewan R, Kaur R, Rajeshwari K. Asymptomatic Cryptococcal Antigenemia in People Living with HIV (PLHIV) with Severe Immunosuppression: Is Routine CrAg Screening Indicated in India? J Assoc Physicians India. 2017;65(4):14-17.
11. Kumar S, Wanchu A, Chakrabarti A, Sharma A, Bambery P, Singh S. Cryptococcal meningitis in HIV infected: experience from a North Indian tertiary center. Neurol India. 2008;56(4):444-9.
12. Meya DB, Manabe YC, Castelnuovo B, Cook BA, Elbireer AM, Kambugu A, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/microL who start HIV therapy in resource-limited settings. Clin Infect Dis. 2010;51(4):448-55.
13. Ford N, Shubber Z, Jarvis JN, Chiller T, Greene G, Migone C, et al. CD4 Cell Count Threshold for Cryptococcal Antigen Screening of HIV-Infected Individuals: A Systematic Review and Meta-analysis. Clin Infect Dis. 2018;66(suppl_2):S152-9.
14. World Health Organization. Rapid Advice: Diagnosis, prevention and management of Cryptococcal disease in HIV -infected adults, adolescents and children. 2011. Available at: whqlibdoc.who.int/ publications/2011/9789241502979_eng. pdf. Accessed on 12 May 2025.

Cite this article as: Kolligouda MG, Khare RL, Dubey P, Sherwani N, Lakra D. The prevalence of asymptomatic Cryptococcal antigenemia in people living with human immunodeficiency virus with severe immunosuppression. Int J Adv Med 2025;12:473-7.