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SERUM CRYPTOCOCCAL ANTIGEN DETECTION IN HIV SEROPOSITIVE INDIVIDUALS

Medical Microbiology

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ABSTRACT

Background and Objective: Cryptococcal infection is the 4th most commonly recognized cause of life-threatening infection among HIV sero positive patients.Cryptococcal infection presents with nonspecific clinical manifestations which contributes to a delay in its diagnosis. The detection of Cryptococcal antigen in the blood represents systemic invasion with the fungus. This is the stage where fungus shows capacity to disseminate to other major organs. Hence, serum cryptococcal antigen (CrAg) serves as an early stage biomarker for detection of disseminated cryptococcosis in HIV patients and can be seen as early as 234 days prior to meningitis symptoms. The main objective of this study was to detect circulating cryptococcal antigen in HIV seropositives and compare with their CD4+ counts. **Methods:** After taking detailed history patient consent was obtained from patients who fulfilled the inclusion criteria. Approximately 3 ml blood was collected and serum was separated. Then the tests were carried on the serum samples for Ag detection by Latex agglutination to detect circulating cryptococcal antigen, as per manufacturer's instructions. **Results:** The incidence of Cryptococcal antigen in our study group was found to be 2.7%. Majority of the cryptococcal antigen positive patients had CD4+ count <200 cell/mm3. **Conclusion:** High morbidity and mortality associated with Cryptococcal meningitis necessitates the importance of its early diagnosis in HIV seropositives

KEYWORDS

AIDS, Cryptococcus neoformans, Cryptococcal antigen, Latex agglutination, Cryptococcal Meningitis

INTRODUCTION

Cryptococcus neoformans (*C. neoformans*) is a ubiquitous encapsulated yeast, it is one of the most common opportunistic pathogen among Human Immunodeficiency Virus (HIV) infected individuals that causes significant infections, which range from asymptomatic pulmonary colonization to life threatening meningoencephalitis.[1]

Although human infections caused by *C. neoformans* were first reported in the early 1900s, the overall number of cryptococcosis cases has been extremely low. Prior to the HIV epidemic, cryptococcosis was an uncommon, systemic infection that affected immunosuppressed patients.[2]

The incidence of cryptococcal meningitis caused by *C. neoformans* has risen markedly over the past 20 years as a result of the HIV epidemic and increasing use of immunosuppressive

Over the years the increase in incidence and severity of cryptococcosis has mirrored the rise of the HIV epidemic, and *Cryptococcus* has become one of the most common causes of fungal pulmonary infections in AIDS patients. In addition, it was the primary cause of death among HIV-infected patients with CD4 counts of less than 100 cells/l before the highly active antiretroviral therapy (HAART) became available.[2]

The administration of highly active antiretroviral therapy (HAART) has resulted in a decrease in the number of cases of AIDS-related cryptococcosis in developed countries, but *Cryptococcus* is still a major problem in developing country where HAART is not readily available.[3]

Cryptococcus neoformans is a heterothallic encapsulated yeast. Microscopically the unicellular cells of the fungus are spherical to oval in shape. Individual cells are surrounded by a polysaccharide capsule. The diameter of the cell can vary from 2-5 µm (capsule deficient) to 30-80 µm in heavily encapsulated cells. It reproduces by budding.[4]

Traditionally, *C. neoformans* was classified into two varieties and five serotypes (A, B, C, D, AD) based on variation in capsular epitopes & different capsular agglutination reaction.[5]

The pathogenesis of cryptococcosis is multifactorial and involves the concerted action of many virulence factors[6], like melanin pigment production, high temperature growth, capsule,urease production etc. Cryptococcus infection is acquired by inhalation so primary organ that is involved is lung.

In immunocompetent individuals, *C. neoformans* infection is either cleared or remains dormant. But in immunocompromised individuals, *C. neoformans* can disseminate into other organs, especially the brain resulting in cryptococcal meningitis (CR).

Globally, cryptococcal meningitis is responsible for 15% of AIDS-related deaths.[7]

On an average the global cryptococcal antigenaemia prevalence is 6·0% among people with a CD4 cell count of less than 100 cells per µL. 9 Cryptococcal antigenaemia is seen highest in countries like D R Congo, Ethiopia, Nigeria with incidence of 15%, 15.5% & 13% respectively.[7]

Untreated cryptococcal meningitis is a fatal disease, infact even with antifungal treatment the outcome is influenced by host factors like any underlying diseases and the fungus. ART can lead to dramatic improvement in the outcome of CR, but the optimal timing of diagnosis of HIV-associated cryptococcal meningitis and of starting ART after the diagnosis is the most crucial is saving the patients life.[8]

A definitive diagnosis of CM is made by positive CSF culture and/or direct identification of the organism by means of India ink staining, it can also be cultured or shown by histopathology as well from different body specimens. A presumptive diagnosis of CM can be made on the basis of a positive cryptococcal antigen (CrAg) using a latex agglutination or an enzyme immunoassay kit. Cryptococcal antigen testing on blood and CSF is a highly reliable and rapid diagnostic technique with excellent sensitivity and specificity.[8]

To know the importance of serum cryptococcal antigen detection, this study was undertaken.

AIMS AND OBJECTIVES:

1. To detect circulating cryptococcal antigen in HIV
2. To compare serum antigen detection with CD4 count.

METHODS

This was a descriptive study where 150 symptomatic HIV sero positive patientsvattending the ART centre attached to Mysore Medical College and Research Institute, between January 2019 to December 2019 were studied. The study was approved by the institutional ethical committee and separate informed consent was obtained from each participant. Study population was determined using the below inclusion and exclusion criteria.

Inclusion criteria:

HIV seropositive patients presenting with symptoms like fever, lethargy, cough with sputum, chest pain, headache, nausea, vomiting, cranial nerve palsies, papilledema, altered mental status.

Exclusion criteria:
1. Any presently diagnosed cryptococcal infection.
2. Any patient with an established diagnosis of any other infection.
3. On anti-fungal treatment
4. Consent refusal

Sample size determination:
Sample size was calculated as 150 considering sensitivity of Latex agglutination as 90% (Sens), prevalence of cryptococcal antigen as 6% (Prev) and 10% error (d), using the formula:

$$n \geq \frac{z_{1-\frac{\alpha}{2}} Sens(1-Sens)}{d^2 x Prev}$$

Sample collection:
After taking informed consent from the patients, detailed history was collected and entered in the performa generated for the purpose. After following total aseptic precaution about 5 ml of peripheral venous blood was collected into sterile vacutainer and was taken to the lab. Then it was centrifuged at 1000xg for 15min. The serum was separated into sterile container.

These serum samples of the patients were subjected for Ag detection by Latex agglutination test, as per the manufacturers instructions. (figure 1 & 2)

RESULTS
Study design: A descriptive study, to know the prevalence of serum *cryptococcal* antigen in 150 HIV seropositive patients. Out of the 150 study population, 84 were males and 66 females. Majority of the patients were in the age group 31-50 years.

The different clinical features presented by the 150 patients were recorded (Table 1). Majority of the patients presented with headache (62%), followed by fever (59%) and cough (54%). (figure 3)

All the patients enrolled in the study were on ART, majority of them were on treatment since 5-10 years. Most patients (27.3%) were on combination therapy of Zidovudine + lamivudine + efavirenz.

It was found that majority (68%) of the subjects in the study had CD4 counts between 200 to 500, whereas 28.6% of the subjects had CD4 counts below 200. (figure 4)

Results of circulating cryptococcal antigen detection test are depicted in table 6 where 2.7% of the total subjects tested positive for cryptococcal antigen. Among the cryptococcal antigen positive patients, majority were male (75%) and most commonly affected age group was of 31-40 years (50%).

Among the positive patients, cough and headache (100%) were the most common presenting feature followed by visual changes and fever. History of tuberculosis was noted in 50% of positive patients. (Table 2)

Majority (75%) of the serum cryptococcal antigen test positive patients had CD4 count below 200, Out of the four cryptococcal antigen test positive patients, one patient had CD4 count of 74 with others having CD4 counts of 191, 187 and 254. (Table 3)

DISCUSSION
Cryptococcus neoformans is the most incriminated fungal pathogen causing meningitis in acquired immune deficiency syndrome (AIDS) patients. This cryptococcal meningitis (CM), a late and disseminated form of cryptococcal infection, leads to substantial morbidity and mortality among HIV-infected individuals. Early in the course of infection, Cryptococococcis in AIDS is usually asymptomatic whereas onset of clinical cryptococococcis in AIDS presents with non specific clinical symptoms as it is found in most pulmonary and meningeal diseases.

Coughing, shortness of breath, fever, sweating, malaise are common presenting symptoms.[9] The detection of Cryptococcal antigen in the blood represents systemic invasion with the fungus.[10] This is the stage where fungus shows capacity to disseminate to major parts of the body. The central nervous system is the commonest site of its dissemination, though cutaneous and adrenal dissemination which are rare are also found in some cases.[11]

Hence, serum cryptococcal antigen (CrAg) is an early stage biomarker for detection of disseminated cryptococcosis in HIV patients and can be seen as early as 234 days prior to meningitis symptoms.[12] Treatment against cryptococcosis should be initiated at the earliest as it is a potentially life threatening illness. C. neoformans related mortality ranges from 10-12% in the developed nations to 50-70% in sub–Saharan Africa. Even after optimal treatment mortality in HIV-associated cryptococcal meningitis has been reported to range from 10 to 25%.[13]

Incidence Of Cryptococcal Meningitis
In a study done by Swati et al in Mumbai, India in 2019 the prevalence of cryptococcaemia in HIV seropositives was found to be 2.67%.74 Prevalence of serum CrAg in persons with advanced HIV infection in London was reported to be 5% in a study by Patel S et al.[14]

According to multiple studies, prevalence of serum CrAg in HIV sero positives of 1% and 19% has been reported in sub-African and Southeast Asian countries.[12] In the current study, the prevalence of cryptococcal antigenemia was 2.70%. This is in correlation with Swati et al study carried out in Mumbai. Many countries have recommended CrAg screening as mandatory in HIV sero positives and early treatment, a few countries like South Africa, Rwanda, and Mozambique, are already implementing such programs.[15] The difference in the prevalence of cryptococcal antigen is probably due to seasonal variation or variable intensity of the presence of cryptococcal species in the local environment.

Age Distribution
In people infected with HIV, the age group in which maximum rate of morbidity (per million total population) is seen is between 31-40 age range.76 In the present study also higher incidence of cryptococcal antigen prevalence was found in the age group of 30-50 age range.

Table 12: Distribution of age groups in various studies

S.No	STUDY	PLACE	YEAR	AGE GROUP
1.	S Manjumder at al	West Bengal	2016	33.17+/-6.63
2.	Lakshmi et al	Hyderabad	2007	20-40 yrs
1.	M R Capoor et al	New Delhi	2007	20-50 yrs
2.	Rakhmanova AG et al	Russia	1999	20-40yrs

Table 13: Sex distribution results of various studies
These results were comparable with other studies which also concluded male preponderance. This predominance can be due to factors like male have more outdoor exposure and females are protected by hormones like estrogen which inhibits growth of Cryptococcus.

S.NO	STUDY	PLACE	YEAR OF STUDY	MALE	FEMALE
1.	Lakshmi et al	Hyderabad	2007	84.62%	15.38%
1.	S P Jaiswal et al	Madhya Pradesh	2002	81.81%	18.19%
2.	Rakhmanova AG et al	Russia	1999	93.30%	6.7%

Clinical Symptoms
Among all the patients who were positive for serum Cryptococcal antigen, all of them had symptoms of cough & headache– 4/4(100%), next common symptom was visual changes- 2/4(50%) followed by fever seen in- 1/4 (25%) and lethargy/weight loss in -1/4 (25%). Two patients among the positives for cryptococcal antigen, had history of tuberculosis. In a study done by S Manjumder et al fever was found in 83% and headache in 100 % of the cases.

Lakshmi et al noted the most common signs and symptoms as headache in 92.31 % and fever in 79.49% of the cases. Vasant et al found headache, altered sensorium and fever in all cases.

Cd4 COUNT
CD4 counts of patients who were positive for CrAg by latex agglutination test were 74, 187,191 and 254. Majority of the patients -

3/4 (75%) had CD4 counts <200 cells/ µl. This result is comparable to results of other studies, 100% of the cases of cryptococcal disease in the studies of Lakshmi et al, Vasant et al, M R Capoor et all had CD4 counts <100 cell/µl whereas in the study conducted by S Majumder et al had 85% cases with CD4 count <100 cell/µl. Incidence of Cryptococcal meningitis is significantly associated with lower Cd4+ count. Majority of the cryptococcal antigen test positive patients were noted to be on combination ART therapy of Zidovudine, Lamivudine & Efavirenz for 5-10 years.

Low CD4 count is a major risk factor for development of C.meningitis. According to WHO all patients with CD4 count <100 cells/µl should be started on prophylactic Fluconazole but drug resistance is a major concern.

CONCLUSION

Among the HIV seropositive patients, Cryptococcal meningitis has emerged as a major cause of mortality and morbidity and one of the most common opportunistic infection throughout the world, particularly in resource-limited countries.

In majority of the cases a period of generalised prodromal symptoms or pulmonary symptoms precedes development of Cryptococcal meningitis in HIV seropositive patients. During this period detection of Cryptococcal antigen can be of great importance in order to prevent development of fatal meningitis.

Our study has shown the prevalence of 2.7% antigen detection in the age group of 30-50 years with common symptoms as headache and cough. Who are on >5years of ART and commonly zidovudine + lamivudine + efavirenz, on this regimen with CD4 count < 200 cells.

These group of people, attending the ART centre, may be screened for antigen detection to give prophylactic therapy for cryptococcal meningitis. To increase the knowledge about this and formulate guidelines, larger group study is needed.

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Conflict of Interest: (Times New Roman 12 Bold)

The authors declare no conflict of interest.

FIGURE 1- Latex agglutination test kit

Figure 2- Latex agglutination test kit reagent

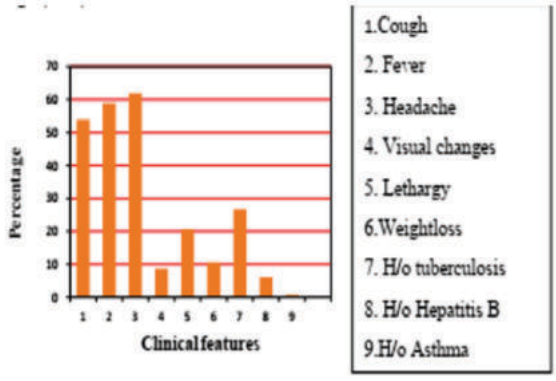

Figure 3- Graph showing different clinical features in the subjects

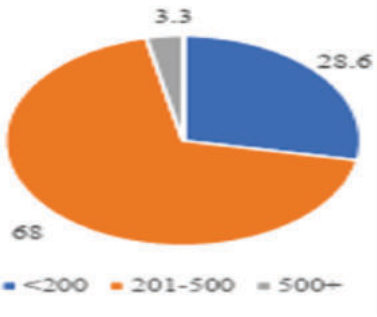

Figure 4- Pie chart showing percentage wise distribution of CD4 counts of all subjects

Table 1- Distribution of study subjects according to age and sex

AGE		SEX		Total
		Male	Female	
<20	Count	0	6	6
	% within sex	0.0%	9.1%	4.0%
21-30	Count	10	20	30
	% within sex	11.9%	30.3%	20.0%
31-40	Count	29	22	51
	% within sex	34.5%	33.3%	34.0%
41-50	Count	26	13	39
	% within sex	31.0%	19.7%	26.0%
51-60	Count	12	4	16
	% within sex	14.3%	6.1%	10.7%
60+	Count	7	1	8
	% within sex	8.3%	1.5%	5.3%
Total	Count	84	66	150
	% within sex	100.0%	100.0%	100.0%

Table 2 – Cross table- distribution of clinical features among positive group

Clinical features	CrAg Test Results	Percentage of feature in positive group
	+ve	
1. Cough	4	100%
2. Fever	1	25%
3. Headache	4	100%
4. Visual changes	2	50%
5. Lethargy	0	0%
6. Weight loss	0	0%
7. H/O Lethargy & Weight loss	1	25%
8. H/O Tuberculosis	2	50%
9. H/O Hepatitis B	0	0%
10. H/O Asthma	0	0%

Table 3- Cross table – showing CD4 counts of patients in the positive group

CD4 Count		CrAg test results	Total in positive group
		+ve	
<200	Count	3	3
	% within cd4_c	6.9%	75%
201-500	Count	1	1
	% within cd4_c	1%	25%
500+	Count	0	0
	% within cd4_c	0.0%	0.0%
Total	Count		4
	% within cd4_c		100.0%

REFERENCES:

1. Bineeta Kashyap, Rajat Jhamb, Shukla Das, Neha Berry, Iqbal R Kaur. Correlation of Cryptococcal Antigen Assay with C-Reactive Protein as Serum and Urine Biomarker in Cryptococcal Meningitis: Experience in a Tertiary Hospital, Journal of Clinical Research in HIV AIDS & Prevention 2013;1(3):12-18.
2. Meng Li and Wanqing Liao. Pulmonary Cryptococcocal Infection, Current Respiratory Medicine Reviews, 2012;8:365-369.
3. Maurimélia Mesquita da Costa1, Francisco Martins Teixeira1, Taysa Ribeiro Schalcher1, Mioni Thielli Figueiredo Magalhães de Brito1. Cryptococcosis, A Risk for Immunocompromised and Immunocompetent Individuals, The Open Epidemiology Journal, 2013;6: 9-17.
4. Spinello Antinori. *Review Article* New Insights into HIV/AIDS-Associated Cryptococcosis, ISRN AIDS, 2013;2013:1-22.
5. Reiko Ikeda,1 Takako Shinoda, Yoshimura Fukazawa, And Leo Kaufman3. Antigenic Characterization of Cryptococcus Neoformans Serotypes And Its Application To Serotyping Of Clinical Isolates. Journal Of Clinical Microbiology.1982 July;16(1):22-29.
6. Thomas R Kozel. Virulence factors of Cryptococcus neoformans. Trends in Microbiology.1995 Aug;3(8):295-299.
7. V Lakshmi, T Sudha, V D Teja, P Umabala. Prevalence Of Central Nervous System Cryptococcosis In Human Immunodeficiency Virus Reactive Hospitalized Patients. Indian Journal of Medical Microbiology. 2007 Jun;25(2):146-149.
8. S. K. Shankar, A. Mahadevan, C. Sundaram, Chitra Sarkar, Geeta Chack, D. N. Lanjewar ,Vani Santosh, T. C. Yasha, V. V. Radhakrishnan. Pathobiology of fungal infections of the central nervous system with special reference to the Indian scenario. Neurology India. 2007 July;55(3):198-215.
9. Favour Osazuwa, John Osilume Dirisu, Patrick Evbaguehita Okuonghae, Ose Ugbebor. Screening for Cryptococcal Antigenemia in Anti-Retroviral Naïve AIDS Patients in Benin City, Nigeria. Oman Medical Journal. 2012 Apr;27(3):228-231.
10. Aberg JA, Watson J, Segal M, Chang LW. Clinical utility of monitoring serum cryptococcal antigen (sCRAG) titers in patients with AIDS-related cryptococcal disease. HIV Clin Trials 2000. Jul-Aug;1(1):1-6
11. Skiest DJ, Hester LJ, Hardy RD. Cryptococcal immune reconstitution inflammatory syndrome: report of four cases in three patients and review of the literature. J Infect 2005. Dec;51(5):e289-e297
12. Swati Vijay, Nayana Ingole , Shashir Wanjare et al. Prevalence of Cryptococcaemia in HIV Seropositive Patients in an Indian Setting. Journal of Clinical and Diagnostic Research. 2019 Jun, Vol-13(6): DC01-DC04
13. Eileen K. Maziarz and John R. Perfect. Cryptococcosis. Infect Dis Clin North Am. 2016 March; 30(1): 179–206.
14. Sheel Patel, Gee Yen Shin, Ishan Wijewardana, et al The prevalence of cryptococcal antigenemia in newly diagnosed HIV patients in a Southwest London cohort. J Infect. 2013 Jan; 66(1): 75–79.
15. Yulin Wang, Philip Aisen, And Arturo Casadevall. *Cryptococcus neoformans* Melanin and Virulence: Mechanism of Action. Infection And Immunity. 1995 Aug;63(8):3131–3136.