A CROSS-SECTIONAL STUDY OF CRYPTOCOCCAL ANTIGENEMIA IN ANTI-RETROVIRAL NAIVE HIV INFECTED PATIENTS

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ABSTRACT

BACKGROUND

Identification and treatment of opportunistic infections in HIV positive patients is as important as antiretroviral therapy (ART) in improving quality of life and increasing their survival. Despite the widespread use of ART, *Cryptococcus* remains a significant pathogen among persons infected with HIV. Screening for this fungus will prevent morbidity, mortality and immune reconstitution inflammatory syndrome (IRIS).

The aim of this study was to screen for *Cryptococcus neoformans* antigen in the sera of ART naive HIV seropositive patients.

MATERIALS AND METHODS

A total of 100 antiretroviral naive HIV positive adults were enrolled in this prospective cross-sectional study. This study was conducted in a tertiary care hospital in Tamilnadu from September 2012 to September 2013. After obtaining written informed consent, serum samples were collected and screened for *Cryptococcus neoformans* antigen using latex agglutination antigen detection kit (CALAS, USA) as per manufacturer's instructions. Basic demographics such as age, sex and CD4 counts were recorded.

RESULTS

Among the 100 HIV positive adults enrolled, 56% were males and 44% were females. Their age ranged from 20 - 70 years with a mean of 40 years; 15% were positive for cryptococcal antigen. Almost 40% of HIV patients with Cryptococcal antigenemia had CD4 counts below 350 cells/mm³.

CONCLUSION

Cryptococcal antigen screening among ART naive HIV positive patients will help in reducing the morbidity and mortality. Early detection and treatment may be the most cost-effective and easily implemented approach to improve the clinical outcomes.

KEYWORDS

Cryptococcus; Cryptococcal Antigenemia; HIV; ART.

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BACKGROUND

The National AIDS Control Organisation (NACO) reported that HIV prevalence at the national level has continued a steady decline from an estimated peak of 0.38% in 2001 - 03 to 0.34% in 2007 and 0.28% in 2012 to 0.26% in 2015. There has been a decline of 67% in estimated new infection since 2000 against a global average of 35%.¹ Since April 2004, the Government of India offers Antiretroviral Therapy (ART) free of charge to eligible patients in ART centres that are present in Government Medical College hospitals and district hospitals. Currently, 1.05 million People Living with HIV (PLHIV) are taking ART through a network of 531 ART centres and 1100 Link ART Centres.¹

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From May 2017, the Government of India has decided to treat all PLHIV with ART irrespective of the CD4 counts, clinical stage, age and population.² The identification and treatment of opportunistic infections is as important as initiating ART in reducing morbidity and mortality in PLHIV. Sometimes, ART can lead to an aberrant inflammatory response and paradoxical clinical worsening known as Immune Reconstitution Inflammatory Syndrome (IRIS).³ This is due to subclinical opportunistic infections and disease exacerbations after starting ART. Therefore, screening for opportunistic infections is important in preventing IRIS.

Cryptococcosis is an important opportunistic infection and a major problem for people with advanced HIV/ AIDS. *Cryptococcus* most commonly infects the central nervous system and lungs of immunocompromised patients and rarely affects optic nerve, bone, liver and lymph node.⁴ Cryptococcal meningitis is one of the most common fungal infections in AIDS patients and has the mortality rate of 25% - 30%.⁵ Worldwide, nearly one million cases of HIV-associated cryptococcal meningitis occur each year, resulting in 625,000 deaths.⁶ Despite the widespread use of Highly Active Antiretroviral Therapy (HAART), *Cryptococcus* remains a

significant pathogen among persons infected with HIV. The incidence and mortality rate of cryptococcosis are extremely high in resource limited settings.⁷ Unfortunately, screening for this fungus prior to the symptoms and infection has been very limited in India. This study was designed to screen for *Cryptococcus neoformans* in the serum of ART naive HIV positive patients.

MATERIALS AND METHODS

This was a prospective cross-sectional study conducted from September 2012 to September 2013. Consenting HIV positive ART naive individuals attending the ART centre at a Government Tertiary Care Hospital in Thanjavur, Tamilnadu were enrolled. Written informed consent was obtained from the study participants after full explanation of the study. Patients on ART and antenatal mothers were excluded from this study. The study was assessed and approved by the Institutional Ethics Committee.

Blood sample (5 mL) was collected by venepuncture under aseptic precautions. Serum was separated and stored at -70°C until further testing. Prior to freezing, 1 mL of serum was transported to the Department of Experimental Medicine of the Tamilnadu Dr. MGR Medical University for screening of *Cryptococcus neoformans* antigen. The test was performed using latex agglutination antigen detection kit (CALAS, USA) as per manufacturer's instructions. Latex particles coated with anticryptococcal globulin reacts with cryptococcal polysaccharide antigen causing visible agglutination. Demographics such as age, gender and CD4 counts were recorded.

RESULTS

Among the 100 HIV positive adults enrolled, 56/100 were males (56%) and 44% were females. Their age ranged from 20 - 70 years with a mean of 40 years $\pm$ 11.8. Fifteen (15/100) of them were positive for cryptococcal antigen (Table 1). The CD4 counts in this study ranged from 121 - 978 cells/mm³ and the median CD4 count was 564 cells/mm³. There was no correlation between Cryptococcal antigenemia and CD4 counts. However, 40% of HIV patients with Cryptococcal antigenemia had CD4 counts below 350 cells/mm³.

Gender	Cryptococcal Antigen Positive	Cryptococcal Antigen Negative	Total	P value
Female	6	38	44	0.785
Male	9	47	56	
Total	15	85	100	

Table 1. Gender-Wise Distribution of Cryptococcal Antigen

DISCUSSION

In 1950s, cryptococcosis was reported in less than 500 patients globally. With the emergence of HIV/ AIDS pandemic, there was an increase in the number of individuals infected with *Cryptococcus*.⁸ Other risk groups for cryptococcosis are transplant recipients on immunosuppressants and those with haematopoietic or other malignancies, advanced renal or liver disease, solid organ malignancy, sarcoidosis, rheumatologic disease and diabetes mellitus.⁹⁻¹¹

Detection of cryptococcal antigen in serum can be used as a presumptive diagnosis of cryptococcal meningitis.¹² An overall prevalence of 15% of cryptococcal antigenemia was

observed in our study. Studies from Nigeria (12.7%),¹³ Ethiopia (14.2%),¹⁴ Congo (15.2%),¹⁵ Bangkok (12.9%)¹⁶ and Uganda (19%)¹⁷ revealed the prevalence of cryptococcal antigenemia was similar to our study. A recent study from South India documented 88.7% of patients with cryptococcal meningitis had HIV infection.⁹ Another study from South India in the year 1996 observed 43.9% of patients with cryptococcal meningitis were HIV positive and reported that there has been a parallel increase in the incidence of HIV and cryptococcal infections.¹⁸ A study from Indonesia documented a weak correlation between CD4 cell counts and the titre of cryptococcal antigen.¹⁹ Whereas in the present study there was no correlation; however, the titre of cryptococcal antigen could not be estimated due to limited resources.

A study from Durban, South Africa estimated 14.7% prevalence of cryptococcal antigen (CrAg) among participants with CD4 > 200/mm³. This was significantly higher than those with CD4 $\leq$ 200/mm³ (7.5%).²⁰ While in the present study, 40% of HIV patients with Cryptococcal antigenemia had CD4 counts below 350 cells/mm³. But as per WHO recommendations, cryptococcal screening is considered prior to ART initiation in patients with CD4 counts of less than 100 cells/mm³. When this recommendation is followed in our setup, many patients with cryptococcal antigenemia are likely to be missed. When ART was initiated in HIV positive patients with undiagnosed cryptococcal disease, they are at risk of developing IRIS and may result in death.²¹ Pre-emptive antifungal treatment can be offered to HIV positive patients with cryptococcal antigenemia that will halt the development of cryptococcal meningitis.

The main limitation of this study was that clinical manifestations were not recorded. Studies with more samples size could have enlightened the importance of cryptococcal antigenemia screening in our setting and especially when ART is offered to all HIV positives.

CONCLUSION

To conclude, this study demonstrated high positivity of cryptococcal antigen among HIV positive ART naive patients. We suggest screening of cryptococcal antigen in all HIV positive patients irrespective of their CD4 counts before starting ART.

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