ORIGINAL RESEARCH PAPER
Volume-8 | Issue-9 | September - 2019 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

OCCURENCE OF CRYPTOCOCCAL ANTIGENEMIA IN HIV POSITIVE CHILDREN: A CROSS-SECTIONAL STUDY

Paediatrics

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ABSTRACT

Background: Cryptococcal antigenemia is strongly associated with the development of cryptococcal meningitis in adults. There is paucity of literature on prevalence of cryptococcal antigenemia in HIV positive children.

Objective: To determine the prevalence of cryptococcal antigenemia in HIV positive children.

Methodology: Observational cross- sectional study was conducted between November 2016 to March 2018 at pediatric HIV clinic of PGIMER and Dr. RML Hospital, Delhi. Clinico- demographic data of HIV infected children between 18 months- 12 years of age was collected. Cryptococcal antigen test by latex agglutination was performed on all of these children.

Results: 100 HIV infected children were studied. Mean age of study population was 9.49 + 3.60 years. 65% were male and 96% acquired the infection through vertical transmission.79% cases were in WHO clinical stage I. Mean CD4 count was 838.4+ 419.17 cells/mm3. All the children were on HAART therapy. No occurrence of cryptococcal antigenemia was found in this study. Therefore, its association with age, sex, nutritional status, WHO stage, CD4 count could not be found.

Conclusion: Cryptococcosis is rare in pediatric HIV. Children with high CD4 counts and on HAART therapy are less likely to develop cryptococcal antigenemia. Further larger scale follow-up studies are required to determine the incidence and risk factors of cryptococcal antigenemia in HIV positive children.

KEYWORDS

INTRODUCTION

Human immunodeficiency virus (HIV) infection is a global pandemic. According to WHO, nearly 36.9 million people world-wide are living with HIV and approximately 0.94 million people died of HIV related illnesses in Globally in 2016, 1.8 million children were living with HIV and 0.12 million children died due to AIDS related illnesses in the same Based on WHO HIV Country Profile 2017, estimated number of people living with HIV in India are approximately 2.1 million, of which 2.9% are children Morbidity and mortality in HIV/AIDS patients is mostly caused by opportunistic infections that occur due to the lowered immune defenses of the patient associated with decreasing CD4 counts. Among various opportunistic infections, meningitis associated with HIV/AIDS has an important impact causing considerable morbidity & mortality. Cryptococcal meningitis (CM) is one of the most common opportunistic infections (OI) among people living with HIV with an estimated 2,23,100 cases of cryptococcal meningitis resulting in 1,81,000 deaths each year (4). It represents a disseminated form of cryptococcal disease with very high mortality rates, prolonged hospitalization and high cost treatment regimens with substantial side effects. Early infection which can be treated with relatively inexpensive drugs like fluconazole may however be not detectable due to lack of symptoms. In 2011, WHO recommended the use of serum cryptococcal antigen test for screening of CM in ART naïve adult patients for early detection and pre- emptive treatment (5). Cryptococcal antigen (CrAg) is a biologic marker of cryptococcal infection, which can be detected in sera, a median of 3 weeks (range 5–234 days) before symptoms of meningitis appear [6]. Studies have shown that otherwise healthy HIV-infected persons with detectable serum CrAg have increased mortality when compared to their CrAg-negative counterparts and pre-emptive treatment of these patients with fluconazole and anti-retroviral therapy (ART) has been shown to improve survival compared with ART alone [7,8].

CrAg detection tests are relatively expensive and hence the use has been limited in resource-limited regions. However, recently, the dipstick CrAg detection test called the lateral flow assay (LFA) has been developed which is relatively cheap, easy-to-use with high sensitivity and specificity [7,9]. The WHO also recommends serum CrAg-based screening for early cryptococcal infection using antigen-based tests, including the LFA [4].

Cryptococcosis in children including immunocompromised children is considered rare; however, there is paucity of literature on the occurrence of cryptococcal antigenemia in children living with HIV (CLHIV) worldwide. The aim of this study was to find out the prevalence of cryptococcal antigenemia in HIV positive children by serum cryptococcal antigen test and to find its association with CD4 count and other clinical and laboratory parameters.

METHODS

This observational cross-sectional study was carried out in the pediatric HIV clinic of our hospital from November 2016 to March 2018 after obtaining institutional review board clearance. All CLHIV follow up with their caregivers are given comprehensive pediatric HIV care including ART. Considering the prevalence of cryptococcal antegenemia to be around 4% to 18% the minimum required sample size with an 8% margin of error and 5% level of significance was 90 patients. One hundred CLHIV between 18 months to 16 years were consecutively enrolled from the clinic after taking written informed consent. One hundred children with documented HIV infection (confirmed by a series of 3 tests as per NACO guidelines) with any CD4 count were included. Children previously diagnosed with cryptococcosis and already on fluconazole or any other antifungal therapy were excluded.

All patients were subjected to detailed history, examination and laboratory investigations. Socio-demographic profile, anthropometry, clinical and laboratory parameters, HIV status (CD4 count) and treatment details of each child were recorded. The clinical records for each CLHIV were reviewed for the mode of acquisition.

Symptoms suggestive of opportunistic infections were asked, specifically any complaints such as fever, vomiting, headache, neck pain, neck stiffness, altered mental status, seizure, photophobia, cough with expectoration and any skin lesions, which is suggestive of cryptococcal meningitis, pulmonary cryptococcosis or cutaneous cryptococcosis. Laboratory data including complete blood count, liver function tests, kidney function tests, calcium, phosphate, CD4 counts

and latex agglutination test for cryptococcal antigen were recorded.

Method of Latex Agglutination Test (Cryptococcal Antigen Detection Test):
CALAS R (Cryptococcal Antigen Latex Agglutination System) was used to detect cryptococcal antigen in the serum. The test is based on a simple agglutination technique to detect capsular polysaccharide of Cryptococcus neoformans, glycuronoxylomonnan (GXM), in the specimen by using coated anti-GXM monoclonal antibody. Agglutination was immediately read and graded from negative to +4. Patient's specimen that had a +2 or greater reaction with either the Detection Latex or Control Latex was titrated with both reagents. A reading of +2 was considered positive.

Data analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. Categorical variables are presented in number and percentage (%) and continuous variables as mean, SD and median. Normality of data was tested by Kolmogorov-Smirnov test. Quantitative variables were compared using unpaired t-test. Qualitative variables were correlated using chi-square test. Univariate and multivariate logistic regression and Pearson Correlation coefficients was planned to find out the risk factors of cryptococcal antigenemia in HIV positive children and assess the correlations respectively. A p value of <0.05 was considered statistically significant.

RESULTS
100 HIV positive children who were regularly attending the pediatric ART clinic were recruited after excluding those who did not meet the inclusion criteria.

Table 1 demonstrates the characteristics of the CLHIV population. The mean age of the cohort was 9.49 $\pm$ 3.60 years. 83 (83%) of them were more than 5 years of age. 65% of the children were boys. Analysis of data from their clinical records demonstrated that, fathers were seropositive for 88 (88 %) of the CLHIV, while mothers were seropositive for 93 (93%) of the patients. Vertical transmission was the most common mode of acquisition of HIV 96(96%), the rest being blood transfusion 3(3%), and unknown in 1 (1%). Nutritional status was normal in 90 (90%) of patients according to WHO BMI growth chart.72(72%)children did not have any symptoms.

Table 2 shows that the majority of children 79(79%) were in stage I of the disease. The mean CD4 count was 838.4$\pm$ 419.17 cells/mm3 and 98 % children had CD4 count more than 200 cells/mm3. All the subjects were on HAART. More than half of the study population was on first line HAART of ZLE or ZLN. In this study population, 91 (91%) of CLHIV were taking ART for less than 10 years and 59 (59%) were not taking cotrimoxazole prophylaxis.

No occurrence of cryptococcal antigenemia was found in this study. Therefore, association of serum cryptococcal antigenemia with age, sex, nutritional status, WHO clinical and immunological stage could not be found.

DISCUSSION:
In our study, none of the patient showed cryptococcal antigenemia. The incidence of cryptococcal meningitis in children with HIV is low. However, it is the most common clinical presentation of cryptococcosis in these children and is a HIV stage 3 defining illness. In the largest of series on cryptococossis, Meiring et al studied the prevalence of cryptococcosis in a population based surveillance. In their study, of the 74 children diagnosed with cryptococcal infection, 67 (91%) were HIV positive. Most of these children (64%) were severely immunosuppressed with CD4 count of less than 50 cells/ul (10). Similarly, in a study from Thailand by Likasitwattanakul et al, 21 HIV-infected children were diagnosed as having cryptococcosis. The 8-year point prevalence of cryptococcosis among hospitalized HIV-infected patients in this study was 2.97%. Sixteen children had cryptococcal meningitis and cryptococcal antigen was positive in sera of all these children (11). In a study from Zimbabve, the prevalence of cryptococcosis among HIV-positive children was reported to be 1.4% [12]. Studies from adult population have shown that early diagnosis and treatment of CM in HIV infected patients decreases the moratlity and morbidity (7,8). CrAg assay is a highly specific and sensitive marker which can aid in early diagnosis of CM.

Similar to our study, Anígilájé et al used cryptococcal antigen lateral flow assay to determine the prevalence of cryptococcal antigenemia in HIV infected children with CD4 count less than 200 cells/ul. Cryptococcal antigenemia was not detected in any of the 88 Nigereian children in this study also (13). Thus our study also supports the WHO recommendation which excludes adolescent and children from routine screening of CrAg.

We had children with a higher mean CD4 count (838.4$\pm$ 419.17 cells/mm^3). This might be one of the reason for absence of cryptococcal antigenemia in our study. However, Anígilájé et al in children with CD4 count <200 cells/mm^{3}and Hajiabdolbaghi et al in adults with CD4 count less than 100 cells/ mm^3 also did not observe cryptococcal antigenemia (0% prevalence) in their cohort (13, 14). Manga et al and Kadam et al found no correlation of CD4 with cryptococcal antigenemia in their studies (15,16). Other researchers have however observed that cryptococcosis is associated with low CD4 counts. In a study by Gonzalez et al, all patients had profound depression of the absolute CD4 counts, history of previous opportunistic infections, and onset of cryptococcosis in the second decade of life (17). Abadi et al also reported CM in children who were profoundly immunosuppressed (median CD4/ cell count, 54/mL) and more than half had previous AIDS defining illness (18).

Thus occurrence of cryptococcal antigenemia in children might be due to a combination of several factors and not be dependent only on CD4 count. HIV infection may increase the risk of cryptococcal infection through several mechanisms like chronic inflammation and immunosuppression. Other possible risk factors that may influence the incidence are age, route of transmission, nutritional status, environmental exposure, duration of HIV infection and HAART regimen used.

The mean age of study population (9.49 years) in our study was similar to study by Anígilájé et al (72.23 ± 41.06 months) and Abadi et al (median age of 9.8 years) The median age at time of diagnosis was around 7 -9 in most of the studies (10,19, 20, 21). In the study from, highest incidence was demonstrated among children with less than 1 year of age and second peak among children in the 5- 10-year age group (10). Overall cryptococcal disease seems to occur less frequently in HIV infected children than in adults with a prevalence of 0.8 to 1.4% (12,17,18) except in Thailand where a point prevalence of 2.97% was observed during an eight-year study among hospitalized HIV-infected patients (11) and in South Africa the incidence of cryptococcosis among HIV-positive children was 47 cases per 100.000 persons (10).

Our study had male preponderance (65 males and 35 females) while in the study by Anígilájé et al, there was almost equal sex distribution ratio (18). The reason for this disproportionate sex ratio in our study could be due to gender bias seen in India with female children often neglected in terms of seeking health care.

Vertical transmission was the major route of transmission (96%) in the current study. Studies have shown that cryptococcal infections were more likely to develop in children with transfusion-associated HIV/ AIDS than in those who acquire the infection via vertical transmission (11,12,18,22).

In a study by Oyella et al in adults, low CD4 (less than 50 cells/mm^3) and low body mass index (15.4 kg/m2 or less) were independent predictors of positive serum cryptococcal antigenemia Goni et al also concluded that low BMI was one of the common predictors of cryptococcal antigenemia in their study (24). Most of our patients had normal nutritional status.

In developing countries, HIV positive children are exposed to all kinds of exposure like farming, pigeon breeding, TB contact etc. Yet, no cryptococcal antigenemia was demonstrated in our study, similar to those of Anígilájé et al and Goldman et al (13,22).

All the cases in our study were on HAART therapy according to NACO guidelines. Few adult studies have suggested that ART might have a

preventive role in cryptococcoccosis (7,8). Ganiem et al study showed that ART was associated with a survival benefit and the benefit was more prominent in the group of patients with cryptococcal antigenemia (risk reduction of 73.3% vs. 58.7%) (8). However, few other adult studies have shown high prevalence of cryptococcal antigenemia in patients on ART (25-27). Also the data from Pediatric AIDS Clinical Trials Group studies before and after the advent of ART indicate that the rate of invasive fungal infection, including cryptococcosis, has remained <0.1 per 100 child-years (28). Therefore, the role of ART in prevention of CM and non occurrence of cryptococcal antigenemia needs to be explored by further research.

There is no data describing the prevalence of cryptococcosis in children in India. This is the first study conducted in our country and second worldwide to determine the prevalence of cryptococcal antigenemia in children. In adult studies it has been shown that, cryptococcal antigenemia (a positive serum CrAg in the absence of clinical disease) at baseline is strongly associated with the development of cryptococcal meningitis and early death (8). Pre-emptive antifungal treatment of positive patients helps in reducing early mortality. Serum CrAg screening of new adult patient is therefore recommended by WHO but not in children and adoloscents. Nevertheless, cryptococcal infection is an important opportunistic infection in HIV infected children which can be prevented by fluconazole treatment.

Our study had some limitations. Firstly, we had limited number of study population with CD4 count less than 100 cells/mm3 in which cryptococcosis usually manifests. Secondly, we need to conduct a larger study in patients not taking ART including or exclusively among patients with low CD4 count (<200 cells/mm3) coming to ART clinics. Thirdly, as this was a cross sectional study we were not able to assess natural history of cryptococcal antignemia.

To conclude cryptococcosis is rare in pediatric HIV. Children with high CD4 counts and on HAART therapy are less likely to develop cryptococcal antigenemia. Further larger scale follow-up studies are required to determine the incidence and risk factors of cryptococcal antigenemia in HIV positive children.

What do we already know about this topic?
Cryptococcal antigenemia, an opportunistic infection is common in HIV infected people and is associated with high morbidity and mortality.

How does your research contribute to the field?
Crytococcal antigenemia is rare in pediatric HIV, more so in children with high CD4 counts and on HAART. Therefore, routine testing for this is not required.

Table 1: Characteristics of study group (n= 100)

Characteristics	Frequency (%)
Age	
18 months- 5 years	17 (17)
6 years - 10 years	42 (42)
11 years - 16 years	41 (41)
Mean Age in Years (S.D)	9.49 ± 3.60
Sex	
Male	65 (65)
Female	35 (35)
Parents with HIV positive status	
Father	88 (88)
Mother	93 (93)
Route of acquisition of HIV	
Vertical	96 (96)
Hematogenous	03 (3)
Unknown	01 (1)
Nutritional Status	
Normal	90 (90)
Obesity	01 (1)
Thinness	09 (9)
Clinical Features	
Symptomatic	28 (28)
Cough	15 (15)
Fever	10 (10)
Hematemesis	1 (1)
Knee Pain	1 (1)
Left Earache	1 (1)
Pain Abdomen	2 (2)
Paleness	1 (1)
Skin Lesion	1 (1)
Vomiting	1 (1)

Table 2: WHO stage, CD4 count and HAART in study group.

Characteristics	Frequency (%)
WHO Clinical Stage	
I	79 (79)
II	10 (10)
III	11 (11)
IV	0 (0)
CD4 Count (cells/mm3)	
<50	0 (0)
50-100	0 (0)
100-200	2 (2)
>200	98 (98)
CLHIV on HAART	100 (100)
Duration of HAART (Years)	
Less than 1 year	4 (4)
1-5 years	55 (55)
6-10 years	32 (32)
More than 10 years	09 (9)
Mean treatment duration (S.D)	4.83±3.50
Types of HAART	
ABC/3TC/LOPI/r	03 (3)
ABC/3TC/NVP	01 (1)
TLE	02 (2)
Z/L/LOPI/r	04 (4)
ZLE	51 (51)
ZLN	39 (39)
Cotrimoxazole prophylaxis	
Yes	41 (41)
No	59 (59)

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