ORIGINAL ARTICLE

FREQUENCY OF CRYPTOCOCCAL ANTIGENEMIA IN HIV TREATMENT NAIVE PATIENTS WITH CD4 COUNT BELOW 200/ML

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ABSTRACT

Background: Cryptococcosis is a dangerous opportunistic fungal infection that poses a significant threat to individuals with advanced HIV/AIDS and compromised cellular immunity. The objective was to determine the frequency of asymptomatic cryptococcal antigenemia in treatment naïve HIV patients.

Material and Methods: This cross-sectional study was conducted at The Indus Hospital and Health Network, Karachi from July to December 2022. We included all consecutive HIV patients of age > 18 years, either gender, who were treatment naïve, with CD4 counts ≤200/µL, and did not have any symptoms of cryptococcal infection. Cryptococcal antigen (CrAg) was tested in blood based on the Direct Agglutination test.

Results: A total of 100 HIV patients were enrolled. The mean age was 35.12 ± 10.93 years. The majority (78%) of the subjects were males, and 2/3rd were sexually active; exposure to birds was identified in 31% of patients. Most of the patients enrolled belonged to WHO HIV stage 1 (61%), followed by stage 4 (30%). The median CD4 count was 103.5 (IQR: 56.5 – 126.5) /µL. 48 patients had CD4 count between 101 to 200/µL, while 51 patients had CD4 count ≤100/µL. Only 2 patients were found to have CrAg positive making an overall prevalence of 2%. Both patients had a CD4 count ≤ 100/µL (n = 2/51, 3.9%). 30% of patients had other opportunistic co-infections (n = 30).

Conclusion: The prevalence of asymptomatic cryptococcosis in the treatment naïve HIV is low in the Pakistani population. A routine CrAg screening can be individualized and avoided in selected patients with CD4 count >100/µL in a resource-limited country like Pakistan.

Keywords: AIDS, HIV stages, Asymptomatic, Opportunistic infection.

BACKGROUND

Cryptococcosis is a severe systemic disease that poses a life-threatening risk to infected individuals. The disease is caused by a group of encapsulated yeast known as Cryptococcus species.[1,2] Cryptococcus is frequently found in the environment, particularly in association with pigeon excreta, soil, and other avian waste products. The pathogen is present worldwide,[3] and is frequently inhaled by individuals. In immunocompetent individuals, cryptococcus generally does not give rise to severe illness. However, in immunocompromised individuals, this fungus can cause a serious opportunistic infection.[4,5] The inhalation of spores from yeast primarily leads to pulmonary infection presenting as symptoms of pneumonia. In individuals who are immunosuppressed, the disease can spread to various other regions of the body, notably the meninges, potentially leading to the development of life-threatening cryptococcal meningitis (CM).[6]

A significant proportion of the population infected with Human Immunodeficiency Virus (HIV) continues to seek healthcare services at a late stage, resulting in acquired immune deficiency syndrome (AIDS).[7,8] This fungal infection affects a considerable proportion of AIDS patients, estimated to be between 60% and 70%.[9,10] Individuals infected with HIV/AIDS who exhibit CD4+ T cell counts below 100 cells/µL are particularly susceptible to Cryptococcosis.[11] Cryptococcal disease is a leading cause of mortality amongst individuals afflicted with HIV in developing regions.[12-15] The incidence of morbidity and mortality resulting from CM exhibits regional variability that is primarily dictated by the prevalence of AIDS.[16] Recent estimates suggest that CM contributes to approximately 15-20% of AIDS-related fatalities worldwide, with the majority of these cases concentrated in the sub-Saharan African region.[8, 13, 15, 17, 18].

Early screening and treatment of cryptococcosis in individuals afflicted with HIV/AIDS is of paramount

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importance.[11] The definitive approach for the identification of cryptococcal disease is predicated on culture obtained from bodily fluids. However, the identification of cryptococcal antigens (CrAg) within the bloodstream is the most frequently utilized technique, with sensitivity and specificity reaching 100%. The CrAg has been observed to exhibit a positive presence in blood for an average duration of 22 days prior to the onset of CM. Additionally, it has been observed that approximately 11% of patients display a positive CrAg test result over 100 days prior to experiencing the onset of CM.[10] There exist multiple procedures for detecting CrAg in cerebrospinal fluid (CSF) or plasma/serum, including latex agglutination (LA), enzyme immunoassay (EIA), and lateral flow assay (LFA) techniques.[7] Screening individuals for subclinical cryptococcal infection upon admission into antiretroviral therapy (ART) programs via point-of-care methods such as CrAg immunoassays exhibits great efficacy in the detection of patients predisposed to the development of cryptococcal meningitis (CM). Early identification facilitates targeted preemptive antifungal therapy to be administered to these individuals, ultimately minimizing the onset of severe illness and mortality.[19] Considering this, we decided to enroll all treatment naïve patients in this study.

The present study sought to determine the frequency of cryptococcal antigenemia in HIV patients with no clinical features of cryptococcal infection on history or examination, with CD4 count below 200/µL. To the best of our knowledge, there has been no research conducted in Pakistan on the cryptococcal antigen rate in HIV patients. Therefore, our research aims to not only assist in gathering relevant regional data but also provide a valuable resource for future references when dealing with individuals affected by cryptococcus among the HIV-infected population within our society.

MATERIAL AND METHODS

This cross-sectional study was conducted at The Indus Hospital and Health Network (IHHN), Korangi campus, Karachi from July to December 2022. Established in 2007, the Indus Hospital, Korangi campus, is a 300-bed state-of-the-art tertiary care facility providing 100% free and quality healthcare to approximately 400,500 patients annually. The IHHN Korangi campus runs an HIV clinic supported through the UNDP Fund, where investigations and anti-retroviral treatment (ART) are provided free of cost to the patients. The sample size was calculated by Open Epi 3.01 using a 95% confidence level, 7.5% margin of error, and 12.7% prevalence of Cryptococcal antigen.[20] Considering the above, the minimum sample size required for this study was 76 patients.

We included all consecutive HIV patients of age > 18 years, either gender, who were treatment naïve, with CD4 counts 200/µL or below. Patients on immunosuppressive medications for any other reason, active malignancy, known history or treatment of cryptococcal infection in the past, any symptoms of cryptococcal meningitis (headache, neck stiffness), and those who received solid organ transplant were not considered eligible.

After approval from the institutional review board, all eligible patients were enrolled in the study. Informed written consent was obtained from patients. Their clinical and demographic data **(**age, gender, absolute CD4 count, ethnicity, socioeconomic status, bird exposure, and comorbidities such as diabetes, and hypertension**)** were entered in a preformed questionnaire followed by Cryptococcal antigen testing in blood. Cryptococcal antigenemia was defined as a positive cryptococcal antigen (CrAg) test in blood, based on Direct Agglutination test using Thermo Scientific™ Remel™ Cryptococcus Antigen Test Kit. Cryptococcus Antigen Test Kit incorporates the use of latex particles sensitized with murine (mouse) IgM monoclonal antibodies. Cryptococcal polysaccharide antigen in patient serum interacts with sensitized latex particles producing visible agglutination. The use of IgM monoclonal antibodies and treatment of serum with protease reduce the potential for false-positive reactions, eliminating the need to perform a companion control latex test to verify the specificity of results.[21,22] Patients were grouped according to their CD4 counts and WHO HIV stages ranging from stage 1 (asymptomatic) to stage 4 (AIDS).[23]

Data were analyzed by using SPSS v26.0. Mean ±SD / Median (IQR) as appropriate were calculated for quantitative variables such as age, years of education, CD4 counts, etc. Shapiro-Wilk test for normality was used to see the data normality. Frequency (n) and percentage (%) were calculated for categorical variables such as gender, ethnicity, comorbidities, etc. Independent t-test was used to compare the means. Chi-square and Fischer Exact tests were used to compare the

baseline characteristics among males vs females. Chi-square test was applied to assess the association of cryptococcal antigen with CD4 count and WHO HIV staging.

RESULTS

A total of 100 HIV patients were enrolled who presented to IHHN during the study period. The mean age was 35.12 ± 10.93 years. The majority (78%) were males. 61% were married; nearly half belonged to the Urdu-speaking ethnicity. 30% were unemployed while 15% were housewives. Exposure to birds was identified in 32% of patients. Most (n = 60, 61%) of the patients belonged to WHO HIV stage 1, followed by stage 4 (n = 30, 30%). Table 1 describes the basic demographic details. The median CD4 count was 103.5 (IQR: 56.5 – 126.5) /µL. 48 patients had CD4 count between 101 to 200/µL, while 51 patients had CD4 count ≤100/µL. Only 2 patients were found to have CrAg positive making an overall prevalence of 2%. Both patients had a CD4 count ≤ 100/µL (n = 2/49, 4.1%). Thirty percent of overall patients had other opportunistic co-infections; of them, tuberculosis was the most common opportunistic infection (21%) followed by syphilis (3%).

Table-1 also provide the comparison of baseline characteristics with gender. Significant difference was observed between employment and marital status among male's vs female's. No difference was observed in education status, comorbidities, bird exposure, opportunistic infections, cryptococcal antigenemia, WHO HIV stage CD4 count among the two genders.

Table-2 describes the association of CrAg HIV stages. None of the patients with HIV stage 1, 2 or 3 was positive for cryptococcal antigen, while cryptococcal antigen positive rate in WHO HIV stage 4 was 6.3%.

Table-3 describes the association of CrAg with CD4 counts. The CrAg positive rate was 3.7% and 4.5% in patients with CD4 count 51-100/µL and ≤50/µL respectively. However, the p value was not significant due to low number of cases.

Table-1: Comparison of baseline characteristics with gender.

		Total n = 100	Male n = 78	Female n = 22	p-value
Age (Years)	Mean ± SD	35.12 ± 10.93	35.22 ±11.5	34.77±8.87	0.867
Ethnicity	Sindhi	8 (8%)	5 (6.41%)	3 (13.7%)	0.528
	Punjabi	38 (38%)	32 (41%)	6 (27.3%)	
	Urdu speaking	51 (51%)	38 (48.7%)	13 (59%)	
	Baloch	1 (1%)	1 (1.28%)	--	
	Pakhtoon	2 (2%)	2 (2.56%)	--	
Education	Illiterate	--	--	--	0.328
	Primary	9 (9%)	8 (10.2%)	1 (4.5%)	
	Secondary	24 (24%)	20 (25.6%)	4 (18.2%)	
	Matric	15 (15%)	12 (15.4%)	3 (13.7%)	
	Intermediate	14 (14%)	8 (10.2%)	6 (27.2%)	
	Graduate	38 (38%)	30 (38.5%)	8 (36.4%)	
Marital Status	Single	37 (37%)	33 (42.3%)	4 (18.2%)	0.005
	Married	61 (61%)	45 (57.7%)	16 (72.7%)	
	Widowed	2 (2%)	--	2 (9.1%)	
Employment Status	Employed	53 (53%)	50 (64.1%)	3 (13.7%)	<0.001
	Unemployed	30(30%)	27 (34.6%)	3 (13.7%)	
	Housewife	15(15%)	--	15 (68.1%)	
	Student	2 (2%)	1 (1.3%)	1 (4.5%)	
Comorbid	Hypertension	1 (1%)	1 (1.3%)	--	0.829
	Diabetes mellitus	2 (2%)	2 (2.6%)	--	
	COPD	1 (1%)	1 (1.3%)	--	
	End stage renal disease	1 (1%)	1 (1.3%)	--	
Bird Exposure	Present	32 (32%)	23 (29.5%)	9 (40.9%)	0.310
Opportunistic	Candidiasis	2 (2%)	1 (1.3%)	1 (4.5%)	0.710
Infection	Tuberculosis	20 (20%)	14 (17.9%)	6 (27.2%)	
	CMV	2 (2%)	1 (1.3%)	1 (4.5%)	
	Pneumocystis	1 (1%)	1 (1.3%)	--	
	Syphilis	3 (3%)	3 (3.9%)	--	

	Toxoplasmosis	2 (2%)	2 (2.6%)	--	
Cryptococcal Antigen	Positive	2(2%)	1 (1.3%)	1 (4.5%)	0.393
WHO HIV Stage	Stage 1	60 (60%)	49 (62.8%)	11 (50%)	0.441
	Stage 2	7 (7%)	4 (5.1%)	3 (13.7%)	
	Stage 3	1 (1%)	1 (1.3%)	--	
	Stage 4	32 (32%)	24 (30.8)	8 (36.3%)	
CD4 Count	≤50	22 (22%)	15 (19.2%)	7 (31.8%)	0.183
	51-100	27 (27%)	23 (29.5%)	4 (18.2%)	
	101-150	34 (34%)	29 (37.2%)	5 (22.7%)	
	151-200	17 (17%)	11 (14.1%)	6 (27.3%)	

Table-2: Relationship of cryptococcal antigenemia with WHO HIV stage.

WHO HIV Stage	Cryptococcal Antigen Positive	Cryptococcal Antigen Negative	p-Value
Stage 1	--	60 (100)	0.227
Stage 2	--	7 (100)	
Stage 3	--	1 (100)	
Stage 4	2 (6.3)	30 (93.8)	

Table-3. Relationship of cryptococcal antigenemia with CD4 count.

	Cryptococcal Antigen Positive	Cryptococcal Antigen Negative	p-Value
151-200	--	17 (100)	0.538
101-150	--	34 (100)	
51-100	1 (3.7)	26 (96.3)	
≤50	1 (4.5)	21 (95.5)	

DISCUSSION

Cryptococcal antigenemia correlates with reduced CD4 counts.[24] Further, its prevalence demonstrates geographical variations.[16] The true incidence and prevalence of CrAg in HIV patients in Pakistan is not known.

In the current study, we showed that in treatment naïve HIV patients, the overall asymptomatic cryptococcal antigenemia rate was only 2%, with CD4 count 200 or below. It is estimated that the annual incidence of cryptococcal infection in Pakistan lies in the range of 501-1000. [16] A retrospective analysis of all HIV/AIDS since 1986 to 1998 including 67 patients reported a rate of 2.5%.[25] Another study from 2012 showed 9% for cryptococcal meningitis.[26] However, these numbers reflect symptomatic patients.

The prevalence of cryptococcosis in HIV patients is high in African countries and varies from 1.7 to 15.8% in sub-Saharan Africa.[16] This high variation within African countries, and low level of cryptococcosis in Pakistan could be attributed to covert clinical and environmental factors affecting Cryptococcus spp growth. Despite the fact that environmental factors are not well elaborated, it appears that Cryptococcus Spp is linked with certain types of soil, flora, and fauna,[27] and exhibit a wide diversity with specific ecological niches.[28] Furthermore, the virulence of the organism also differs based on lineage and species.[29]

The rate of cryptococcal antigenemia appears to increase significantly with drop in CD4 count. The global prevalence of cryptococcal antigenemia is estimated to be around 2% in adults with CD4 counts between 101 – 200 /µL.[30] However, it increases to approximately 6% for patients with CD4 counts ≤100 /µL. (16) One study conducted in Indonesia found that among ART naïve, asymptomatic, newly diagnosed HIV-infected adult outpatients with CD4 T-cell counts below 100 cells/µL, the prevalence of cryptococcal antigenemia was 7.1%.[31] In a recent metanalysis, the pooled prevalence of cryptococcal antigenemia was 6.5% (95% CI, 5.7%–7.3%) in individuals with CD4 count ≤100 cells/µL and 2.0% (95% CI, 1.2%–2.7%) in those with CD4 count 101–200 cells/µL. In our study, the cryptococcal antigenemia rate was 4% in patients with CD4 count <100/µL. None of our patients with CD4 count >100/µL had Cryptococcal antigenemia.

HIV stage is also correlated with cryptococcal antigenemia. As the HIV infection progresses to advance stages, the likelihood of developing cryptococcal antigenemia becomes greater. In a study from Malwai, the prevalence of cryptococcal antigenemia among patients with WHO clinical HIV/AIDS stage 3 was 1/80 (1.3%) and with WHO

clinical HIV/AIDS stage 4 was 1/20 (5.0%). In our study, both of our cryptococcal antigen positive patients belonged to WHO HIV stage 4, making the prevalence of 6.9% in this group. None of the patients with HIV stage 1, 2 or 3 was positive for cryptococcal antigen.

In our study, both patients with positive CrAg had CD4 count <100 cells/µL. Since the numbers identified were low, no other demographic factors like age or gender could be established, which is consistent with previous studies.[32, 33] Gender-specific differences in HIV have been reported.[34] The majority of our patients were male, in contrast to some previous reports of female preponderance of HIV in African countries.[35] Both of our patients were started on antifungal treatment after ruling out central nervous system involvement by cerebrospinal fluid examination, followed immediately by ART for HIV.

STRENGTHS OF THE STUDY

IHHN runs a dedicated HIV clinic with a large catchment area. Since IHHN provides free diagnostics and treatment, the ease of testing made it possible for us to undertake the study. As far as we could research, this was the first study of its kind from Pakistan.

LIMITATIONS OF THE STUDY

Our study was limited by single center design; we were unable to repeat tests or follow patients longitudinally to detect false negatives; given the low number of CrAg-positive patients, we were unable to perform risk analysis. Finally, we did not check HIV viral load, hence could not assess its relationship with cryptococcal antigenemia.

CONCLUSION

The prevalence of asymptomatic cryptococcosis in the treatment naïve HIV is low in the Pakistani population. A routine CrAg screening can be individualized and avoided in selected patients with CD4 count >100/µL in a resource-limited country like Pakistan.

AUTHOR CONTRIBUTION:

Noorulsaba Shaikh: Conception of the work, acquisition, analysis, and interpretation of data, and drafting the manuscript.

Naseem Salahuddin: Participated in design of the work and critical revision for important intellectual content

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