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Prevalence of Cryptococcal Antigenemia and Its Correlation with Clinico-Immunological Status in HIV Infected Patients

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Abstract: *Cryptococcosis is one of the most common and life threatening opportunistic infection in HIV infected patients. Although incidence of cryptococcal infection has decreased in the era of highly active anti retroviral therapy (HAART), it still remains an important cause of morbidity and mortality in the AIDS population, especially in the developing world. This cross-sectional, observational study was conducted in the Department of Medicine & CoE ART centre in collaboration with Department of Microbiology, RIMS, Imphal for a period of two years to determine the prevalence of cryptococcal antigenemia in HIV infected patients and to correlate cyptococcal antigenemia and clinico-immunological status of HIV infected patients. The study revealed that the prevalence of cryptococcal antigenemia among the HIV infected patients irrespective of their ART status was 9.06%. Mean CD_4 count among the CALAS positive study participants was 73.75±44.85 cells/µL and 68.9% of them had CD_4 count <100 cells/µL. The finding of this study may help to adopt the assessment of cryptococcal antigen as a secondary preventive study in health programme by recommending pre-emptive anti-fungal therapy and in formulating Cryptococcus preventive strategy for HIV infected patients.*

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I. Introduction

Cryptococcus is a leading mycological cause of morbidity among HIV-infected patients. In many patients, it is the first indication of AIDS. Cryptococcus neoformans is an encapsulated yeast-like fungus. The spectrum of cryptococcal infection ranges from asymptomatic colonization to pulmonary cryptococcosis, cryptococcal meningitis and severe disseminated disease.[1] Diagnostic methods include microscopy and culture or laboratory based Cryptococcal Antigen testing, such as Enzyme Immunoassay and Latex Agglutination. More recently, Lateral Flow Immunoassay was introduced as an alternative to CrAg testing.[2] The World Health Organisation recommends routine screening for cryptococcal disease in Antiretroviral Treatment (ART) naïve adults with a CD4 cell count <100 cells/µL prior to ART initiation in populations with a high prevalence of cryptococcal antigenaemia.[3] Untreated, cryptococcal meningitis is fatal. The recommended initial treatment for acute disease is Amphotericin B for 2 weeks, followed by Fluconazole alone for an additional 8 weeks. Early diagnosis and treatment reduces mortality and morbidity. Cryptococcal antigen screening in HIV patients can be an important tool in this aspect and thus preventing development of severe form of the disease.[4] Manipur being one of the high prevalence states for HIV infection in India, finding of this study may help to adopt the assessment of cryptococcal antigen as a secondary preventive study in health programme by recommending pre-emptive anti-fungal therapy. It will also contribute immensely in formulating Cryptococcus preventive strategy for HIV infected patients at large.

Aims and objectives:

1) To determine the prevalence of cryptococcal antigenemia in HIV infected patients.
2) To correlate cryptococcal antigenemia and clinico-immunological status of HIV infected patients.

II. Materials and Methods

Study setting: Department of Medicine, CoE ART Centre RIMS, in collaboration with Department of Microbiology, Regional Institute of Medical Sciences (RIMS), Imphal.

Study design: Cross-sectional, Observational study.

Study period: This study was carried out for a period of 2 (two) years from September 2016 to August 2018.

Study population: HIV positive patients with or without Antiretroviral therapy (ART) admitted in Medicine ward or patients attending Medicine OPD, CoE ART Centre, RIMS Imphal.

Inclusion criteria:
1. Adults 15 years of age or older.
2. All sexes.
3. HIV patients with or without ART.

Exclusion criteria:
1. Those not willing to participate in the study.
2. History of systemic anti-fungal therapy.
3. Terminally ill patients.
4. History of Chemotherapy/ Malignancies/ patients on immunosuppressants.

Sampling: Consecutive cases of HIV infected patients attending Medicine OPD/ Ward and CoE ART Centre, RIMS, Imphal, satisfying the inclusion criteria and giving due consent and/or accent were enrolled.

Sample size: A sample size of 144 was arrived by using the formula $\frac{\mathbf{4PQ}}{\mathbf{L^2}}$
where, P is prevalence = 10% (taken from a study by Devi SB et al[5])
Q = 100-P = 90
L is the absolute allowable error = 5% at 95% confidence interval.

Study Variables:
1. Confirmation of HIV: Diagnosis of HIV infection was done as per the NACO Guidelines using ELISA/ Rapid Kit.
2. Cryptococcal antigen detection: The detection of the Cryptococcal antigen in this study was done by CALAS (Cryptococcal Antigen Latex Agglutination System) developed by Meridian Bioscience, Inc. It is a qualitative and semi-quantitative test system which detects capsular polysaccharide antigens of Cryptococcus neoformans in serum and cerebrospinal fluid. It is both sensitive as well as specific and is proven to be superior to India ink mount. CALAS utilizes latex particles coated with anti-cryptococcal globulin (Detection Latex). The Detection Latex reacts with the cryptococcal polysaccharide antigen causing a visible agglutination. Latex particles coated with normal globulin act as one of the control reagents. The gradation of the reaction strengths are as follows:
- Negative (-): a homogenous suspension of particles with no visible clumping.
- 1+: Fine granulation against a milky background.
- 2+: Small but definite clumps against a slightly cloudy background.
- 3+: Large and small clumps against a clear background.
- 4+: Large clumps against a very clear background.

3. CD4 count: CD4 cell count was done by automated analyser, Fluorescence Activated Cell Sorter (FACS) as per the NACO guidelines.
4. Clinical staging: Clinical staging of the patient was done as per WHO guidelines.

Procedure:
- Study was carried out with the clearance from the Research Ethics Board.
- Informed written consent was taken from the patient/ patient party.
- All the selected patients were subjected to comprehensive questionnaire/ history taking/ thorough detailed examination (as given in Annexure-I).
- All the routine examination was done as per NACO recommendation.
- Blood and CSF sample was sent for Cryptococcal antigen detection. Other relevant sample (tissue/ body fluid) was sent for detection of Cryptococcus (HPE, India Ink preparation, Culture etc.). At the same time, another blood sample was sent for CD4 count.
- All the data collected were documented and analyzed statistically to draw a useful conclusion.

A total confidentiality was maintained by coding of patient's data throughout the study.

Approval of Research Ethics Board: All the participants were informed about the nature of the study which was fully explained in participant information sheet and those who agreed to participate were required to sign in informed consent form. Ethical approval for this study was obtained from Research Ethics Board, Regional Institute of Medical Sciences, Imphal.

Statistical analysis: Descriptive statistics like mean, standard deviation and proportions was used. Independent samples t test and multivariate linear regression analysis were performed for result and analysis. P value less than 0.05 was taken as a level of significance. All the data collected were analysed using a commercially available software SSPS (Statistical Package for the Social Sciences software).

III. Results

Out of 850 study participants, 77 (9.06%) were found to have cryptococcal antigen (CALAS) positive either in serum or CSF. Mean age of the study participants was 37.74±10.53 years. 46 (59.7%) were male and 31 (40.3%) were female. Majority of CALAS positive study participants presented during the rainy season (42.9%) and least in winters (6%).

Duration of presenting signs and symptoms ranged from 1 week to 4 months, with majority presenting after 4 weeks (32.5%).Out of 77 CALAS positive study participants, 1 (1.29%) participant did not have any sign or symptom and was incidentally found to be CALAS positive. Lethargy (90.9%) was the most common presenting complaint followed by headache which was seen in 68 (88.3%) participants. 48 (62.3%) presented with complaint of fever and vomiting. Signs of meningeal irritation (neck rigidity, Kernig's and Brudzinki's sign) was seen 39 (50.6%) participants while other neurological manifestations like altered sensorium, seizure, limb paralysis, slurring of speech etc. was seen in 34 (44.2%) participants. Only 8 (10.4%) participants complained of cough. 6 (7.8%) participants developed skin lesions and 3 (3.9%) had lymphadenopathy.

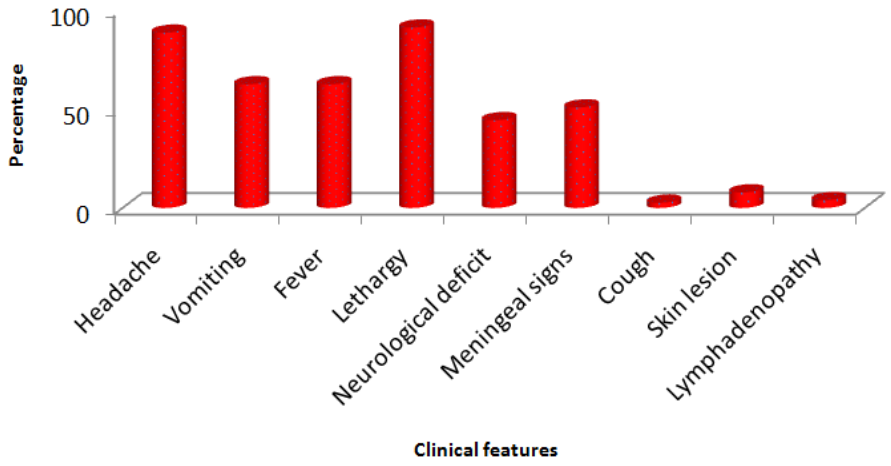

Figure 1: Clinical features distribution of the CALAS positive study participants

46 (59.7%) of the study participants who were found be CALAS positive were ART experienced while the rest 31 (40.3%) were ART-naive. Of the 46 ART experienced participants, 39 (84.8%) were on first line ART and 7 (15.2%) were on second line ART regime.

	No. of patients (n=77)	**%**
ART status		
• Experienced	46	59.7
• Naive	31	40.3
ART treatment regime	N=46	
• 1st line	39	84.8
• 2nd line	7	15.2
• 3rd line ART	0	0.0

Table 1: Distribution of CALAS positive participants based on ART status and regime

Majority of the CALAS positive study participants (59%) who were on ART had poor adherence to the ART i.e. missing more than 3 doses a month (<95% adherence).

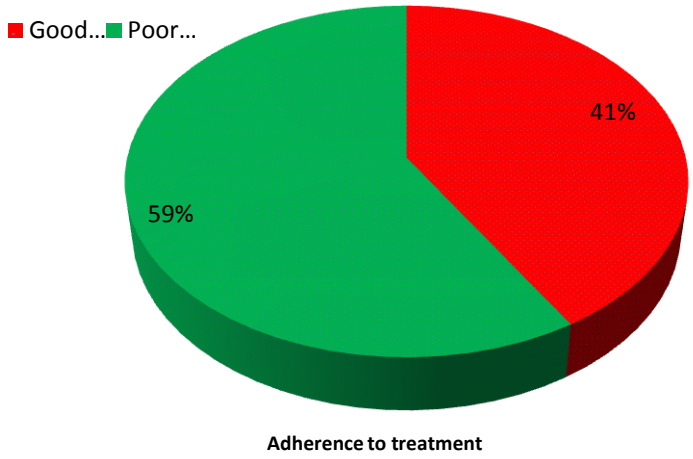

Figure 2: Adherence to treatment distribution of CALAS positive study participants (N=46)

Of 77 CALAS positive study participants, serum CALAS was done in 66 participants of which 59 (89.4%) were positive. CSF CALAS report was available for 73 CALAS positive participants of which only 1 (1.4%) was negative.

	No. of patients	**%**
Serum CALAS	(n=66)	
• Negative	7	10.6
• 1+	15	22.7
• 2+	32	48.5
• 3+	12	18.2
• 4+	0	0.0
CSF CALAS	(N=73)	
• Negative	1	1.4
• 1+	7	9.6
• 2+	39	53.4
• 3+	26	35.6
• 4+	0	0.0

Table 2: Serum and CSF CALAS findings of CALAS positive participants

India ink preparation was positive for cryptococcus species in 71 (92.2%) CALAS positive study participants. Fungal culture from different body fluids and tissue was positive for cryptococcus species in 64 (83.1%) of the study participants.

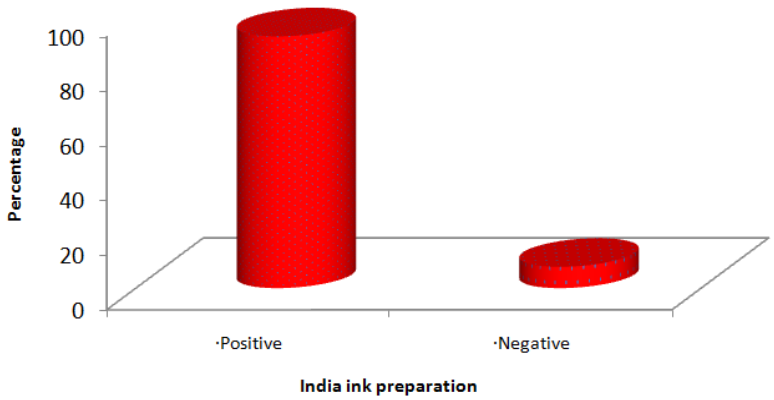

Figure 3: India Ink preparation findings of CALAS positive participants

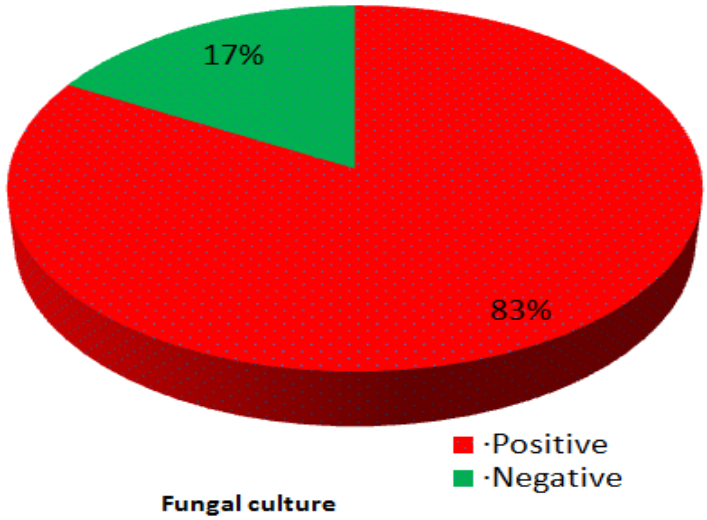

Figure 4: Fungal culture findings

Among the 77 CALAS positive participants, majority i.e. 29 (37.7%) had CD_4 counts of less than 50 cells per µL followed by 24 (31.2%) in CD_4 count range 50-100 cells per µL and 20 (26.0%) in CD_4 count range 100-150 cells per µL. 3 (3.9%) participants had CD_4 count in range 150-200 cells per µL and only 1 (1.3%) had CD_4 counts more than 200 cells per µL. CD_4 count ranged from 2 to 225 cells per µL with a mean of 73.75±44.85 cells per µL.

CD4 count in cells per microlitre	No. of patients (n=77)	%
• <50	29	37.7
• 50-100	24	31.2
• 100-150	20	26.0
• 150-200	3	3.9
• >200	1	1.3

Table 3: Distribution of CALAS positive participants based on CD_4 count

Imaging of brain (CT/MRI) was done in 38 CALAS positive study participants of which 29 (76.3%) had normal study. 9 (23.7%) study participants had abnormal brain imaging. The abnormal findings include infracts, encephalomalacia, demyelination changes, cerebral atrophy, ring enhancing lesions and cerebral edema.

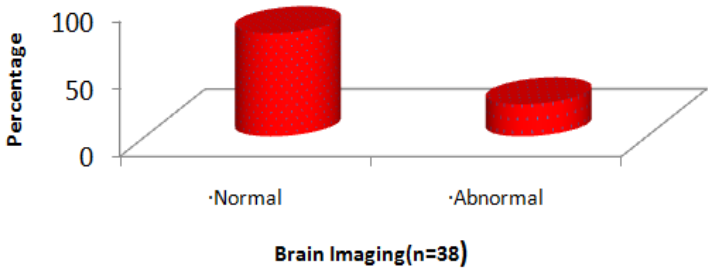

Figure 5: Brain imaging study of CALAS positive study participants

CSF analysis was done in 73 study participants who were CALAS positive and were suspected to have CNS cryptococcosis. CSF protein was raised i.e. more than 50 mg/dL in majority 70 (95.9%) of study participants; normal i.e. 15-50 mg/dL in 2 (2.7%) and low in only 1 (1.4%) study participant. Low CSF sugar i.e. less than 40 mg/dL was seen in 42 (57.5%) study participants and normal i.e. 40-70 mg/dL in remaining 31 (42.5%) participants. Majority 70 (95.9%) participants had raised total cell count in CSF i.e. more than 5 cells

per mm^3 and only 3 (4.1%) had normal CSF total cell count. All the study participants (n=73) had predominant lymphocytic picture in CSF analysis.

	No. of patients (n=73)	**%**
CSF Protein		
• Normal	2	2.7
• Raised	70	95.9
• Low	1	1.4
CSF Sugar		
• Normal	31	42.5
• Raised	0	0.0
• Low	42	57.5
CSF Total Cell count		
• Normal	3	4.1
• Raised	70	95.9
• Low	0	0.0
CSF Cell type		
• Predominant Lymphocytes	73	100.0
• Predominantly polymorphs	0	0.0
• Mixed	0	0.0

Table 4: Cerebrospinal fluid (CSF) findings of CALAS positive participants

Tissue fine needle aspiration cytology (FNAC) or biopsy was done in 9 CALAS positive study participants of which 4 (44.4%) showed features of Cryptococcus i.e. presence of capsulated budding yeast cell. 3 were seen in skin biopsies while 1 was seen in lymph node FNAC.

Tissue FNAC/ Biopsy	**No. of patients (n=9)**	**%**
• No features of Cryptococcus	5	55.6
• Features of Cryptococcus	4	44.4

Table 5: Tissue FNAC or Biopsy findings of CALAS positive study participants

Among the 77 CALAS positive study participants, maximum i.e. 41 (53.2%) received only Amphotericin-B followed by Fluconazole. 19 (24.7%) received Amphotericin-B along with Flucytosine followed by oral Fluconazole. Other treatment like Voriconazole were given to 3 (3.9%) participants. 7 (9.1%) did not complete the treatment while rest 7 (9.1%) did not receive the treatment at all.

Treatment	**No. of patients (n=77)**	**%**
• No treatment	7	9.1
• Incomplete treatment	7	9.1
• Only Amphotericin_B followed by Fluconazole	41	53.2
• Amphotericin B & Flucytosine followed by Fluconazole	19	24.7
• Others	3	3.9

Table 6: Distribution of CALAS positive study participants based on treatment received

Majority i.e. 60 (77.9%) of the study participants improved with the treatment while in 7 (9.1%) there was no improvement or the study participant expired. 9 (11.7%) study participant did not complete the treatment or had left the hospital against medical advice before the completion of treatment. One study participant was asymptomatic and was not initiated on any treatment as he was incidentally found to be serum CALAS positive during the routine follow up.

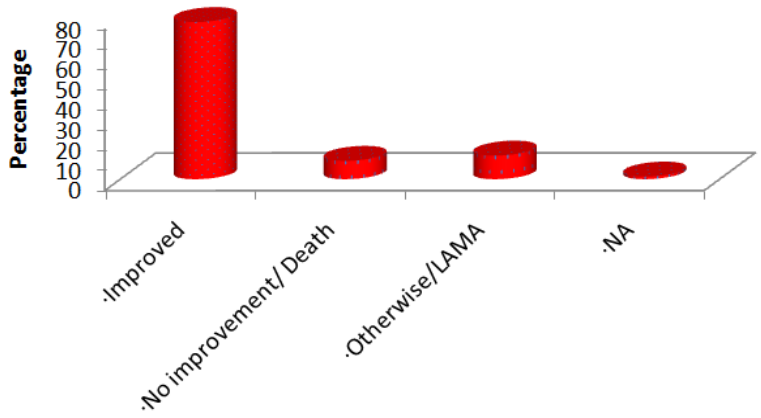

Figure 6: Distribution of the CALAS positive study participants based on the treatment response or outcome (n=77)

IV. Discussion

Cryptococcosis constitutes as one of the most common and life threatening opportunistic infection in HIV infected patients. Although incidence of cryptococcal infection has decreased in the era of highly active anti retroviral therapy (HAART), it still remains an important cause of morbidity and mortality in the AIDS population, especially in the developing world. This cross-sectional observational study was conducted in 850 study participants who were HIV positive patients with or without anti-retroviral therapy (ART) admitted in Medicine ward or attending Medicine OPD, CoE ART centre, RIMS, Imphal with the objective to determine the prevalence of cryptococcal antigenemia in HIV infected patients and to correlate cryptococcal antigenemia and clinico-immunological status in HIV infected patients. In this study, the prevalence of cryptococcal antigenemia was 9.06% (77 out of 850). An study conducted by Kadam D et al[6] in 2017 showed 8% prevalence of cryptococcal antigenemia amongst adult HIV patients in Pune, Maharashtra. Most of the study participants who were CALAS positive (35.1%) were in the age group of 31-40 years and the mean age was 37.74±10.53 years. Males were infected predominantly compared to females constituting 59.7% of the CALAS positive study participants. In the study conducted by Anuradha S et al[7] in 2016 had similar finding where the mean age of the study subjects was 36.2±9.48 years and 73.4% were men. Majority (42.9%) of the CALAS positive study participants presented between the month of June and August which is characterised by high temperature and high relative humidity. Kuroki M et al[8] in 2004 showed high isolation rate of cryptococcal species i.e. 28% during the summer and rainy season from HIV endemic region in Thailand. The duration of the presenting signs and symptoms ranged from 1 week to 4 months with majority (32.5%) presenting after 4 weeks. The most common presenting symptoms were lethargy (90.9%) and headache (88.3%) followed by fever (48%) and vomiting (48%). Meningeal signs and neurological deficits were seen in 39% and 34% of study participants respectively. Skin lesions were seen in 6% of the CALAS positive study participants. 1 (1.29%) participant did not have any signs or symptoms and was incidentally found to be CALAS positive. Study conducted by Kapila K et al[9] in 2003 reported that duration of symptoms ranged from 10 days to 6 weeks (average 20 days) with common presenting signs and symptoms being headache (85.7%), generalised weakness (71.4%), fever (42.8%), nausea and vomiting (42.8%), neurological deficits (28.5%) and meningeal signs (14.2%). Another study conducted by Antinori S[10] in 2013 showed that 5.9% of the study subjects with cryptococcal antigen positivity had skin lesions. In 2016 Vidal J et al[11] showed their study that 3.1% of the subjects were asymptomatic cryptococcal antigen positive. In this study, most of the CALAS positive study participants were newly detected HIV (41.6%). Majority of the CALAS positive study participants were ART experienced (59.7%) of which maximum (59%) were had poor adherence to ART. Similar finding was demonstrated in a study conducted by Vidal J et al[11] in 2016 were 74% were ART experienced with irregular use and/or having defaulted from ART. Out of 66 serum CALAS reports of the CALAS (serum or CSF) positive study participants, 89.4% were positive. Also, out of 73 CSF CALAS report, 72 (98.6%) were positive. Kaufman L et al[12] reported in their study reported that cryptococcal antigen was detected in CSF specimens in 99% of cases but in only 87% of serum samples. In this study, India ink preparation was positive in 92.2% and fungal culture was positive in 83.1% of the study participants who were CALAS positive. A comparative study conducted by Saha D et al[13] in 2009 reported India ink positivity rate of 93.4% and fungal culture positivity rate of 95.6% while assessing the sensitivity and specificity of different diagnostic test for Cryptococcus in CSF of HIV positive patients. The mean CD_4 count in this study was 73.75±44.85 cells per µL and 68.9% of the CALAS positive study participants had CD_4 count of less than 100 cells per µL. In a study conducted to study the cost

effectiveness of cryptococcal antigen screening to prevent deaths among HIV infected persons, Meya D et al[14] in 2015 found that the mean cohort CD_4 count was 79 cells per µL and 51% patients having CD_4 count less than 100 cells per µL. It this study, brain imaging study (CT/MRI) was abnormal in 23.7% study participants who were CALAS positive. A comparatively higher value was seen this study compared to a study conducted by Moosa M et al[15] where CT scan of brain revealed abnormalities in 15% of study subjects after cerebral atrophy was excluded as an abnormality in their study. It has been observed that there was significant association between CSF findings (Protein, Sugar & Cell counts) and CSF CALAS positivity and its grading. CSF findings were within normal values for those with CSF CALAS negative but were altered as the CALAS grading increased. Majority of study participants who had grading other than negative had raised CSF protein (95.9%), low CSF sugar (57.5%), raised total CSF cell count (95.9%) and Lymphocytic predominance (100%). Devi SB et al[5] in 2013 studied the profile of cryptococcal meningitis in AIDS patients found that majority of study participants had increased CSF protein level (134.47±98 mg/dL), low CSF sugar (37.68±20.8 mg/dL), raised total CSF cell count (28.26±2.8 cells) and a lymphocytic predominance (92%). An another study conducted by Moosa M et al[15] revealed similar CSF findings i.e. elevated protein (81%), low sugar (61%) and raised cell count with lymphocytic predominance (54%). To calculate the prevalence of cryptococcal antigenemia in the present study, sample size was calculated by taking the prevalence of cryptococcal meningitis in AIDS patients from a previous regional study, so limitation may be there due to relatively inadequate sample size. Due to logistic problems, HIV viral load for every patient at the time of presentation and species identification of the Cryptococcus could not be done by the researcher. Being a cross-sectional study, possibility of recall bias could not be ruled out. There is paucity of data; at least in this region about the prevalence of cryptoccocal antigenemia in HIV infected patients. The outcome of the study could pave the way for formulating viable ways and means for enabling early diagnosis and treatment of cryptococcosis in HIV infected patients thus reducing its morbidity and mortality.

V. Conclusion

Prevalence of cryptococcal antigenemia among the HIV infected patients irrespective of their ART status was 9.06%. Majority of them were male between the ages of 30 to 40 years. Headache and lethargy were the most common presenting complaint. 1.29% of study participant did not have any signs or symptoms. Most of the study participants were ART experienced with poor adherence to ART. 89.4% were serum CALAS positive and 98.6% were CSF CALAS positive. India ink preparation positivity was 92.2% while the fungal culture positivity was 83.1%. Mean CD_4 count among the CALAS positive study participants was 73.75±44.85 cells/µL and 68.9% of them had CD_4 count <100 cells/µL. Findings of this study emphasises that screening for cryptococcal antigen has a substantial role for the early detection of cryptococcal infection in HIV infected patients and routine screening of cryptococcal antigen should be performed in HIV patients CD_4 count of less than 100 cells per µL.

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