

CSF Study in 101 Cases of Neuro AIDS In Tertiary Hospital

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Background: The important causes of death from HIV/AIDS are opportunistic infections and cancers both of which are frequently the result of the progressive failure of the immune system.^{1,2} Tuberculosis co-infection is one of the leading causes of sickness and death in patients of HIV/AIDS and causing 25% of HIV related deaths.³

Hepatitis C is another common co-infection where each disease increases the progression of other disease.⁴ Programmes encouraging the abstinence don't affect the subsequent risk of HIV.⁵ Comprehensive sexual education provided at high school may decrease high risk behaviour.⁶

Up to 70% of infected individuals have neurological symptoms, and meningitis is the most common cause of HIV related mortality and morbidity in maximum number of patients.⁷ In India according to some of the studies tubercular meningitis is the most common neurological manifestation in HIV patients, followed by Cryptococcus meningitis.

The clinical diagnosis of central nervous system (CNS) diseases in patients with AIDS is based on neurological examination and Neuro imaging studies. Further examinations, such as standard analysis of cerebrospinal fluid (CSF) may be useful. The diagnosis of pathogen in the CSF or brain makes a diagnosis of etiology possible.

Hence, the present study was undertaken to evaluate the CSF of patients suspected of Neuro AIDS.

I. Material And Methods

This was a hospital based cross sectional study, conducted for a period of 2 years. Total of 101 cases were studied. All HIV positive patients with neurological signs and symptoms irrespective of age and sex or whether receiving or not receiving ART were included in this study. CSF tap of these patients were done. The study was carried out in the department of pathology. HIV positive patient without neurological signs and symptoms were not included in this study

Methods of Collection Of Data

A detailed history of patients like age, sex, address, family history, mode of transmission, treatment history and history of coexisting illness were taken and entered in proforma. Following tests were done by the physician to diagnose HIV positive patient with neurological complications ie:

- ELISA/Tridott/Abott
- CD4+ count
- VDRL
- HBSAg
- CT/MRI

CSF tap from diagnosed Neuro AIDS patients was done by lumbar puncture under all aseptic precautions and sent for study in pathology department.

Once the CSF received, it was reported under following headings:

- Gross appearance, Cell count, Biochemistry(Sugar, Proteins), Stains (Gram stain, AFB and India ink)

Reporting Of CSF

Gross appearance of CSF was reported accordingly. As it varies in different conditions. Direct wet mount of CSF was taken for microscopic examination. One drop of CSF after centrifugation was taken directly on a glass slide covered with a cover glass, and was examined under the microscope.

Cell Count in CSF

TLC

Cell count on CSF was done manually on undiluted sample on a counting chamber

Procedure

CSF sample was properly mixed and the counting chamber was covered with a cover slip. The counting chamber was filled with a fluid and allowed to stand for 2 minutes for the cells to settle down. Fuchs Rosenthal counting chamber was used for counting the cells in CSF. In case of undiluted CSF, total number of cells counted in 5 squares represented the total count per mm³ of CSF.

DLC

It provided with the information of relative proportion of various leukocytes. If the CSF contained few cells then it was centrifuged at high speed for 10 minutes and a smear was made from the sediment. If CSF contained many cells, then a smear was made directly from the uncentrifuged sample, stained with the romanowsky stain (Leishman stain), and examined under the microscope.

Chemical Examination Of CSF

The routine examination of CSF for sugar was carried out in biochemistry laboratory with the help of VITA LAB - Selectra E machine and CSF proteins was carried out with the help of prietest Micro Protein reagent on photometric system in biochemistry laboratory. AFB, gram staining and culture was also performed on CSF to look for Mycobacterium tuberculosis. The culture reports were collected from the microbiology department.

Tubercular meningitis was diagnosed on the basis of existing criteria.⁸

- Definite- demonstration of M. tuberculosis (AFB) in CSF.
- Highly probable- M. tuberculosis not demonstrated in the CSF but
- CSF findings compatible with TBM ie proteins>0.60g/l, sugar<60% of corresponding blood sugar and >20cells/mm³ with lymphocytic predominance.
- Evidence of extra central nervous system (CNS) tuberculosis.
- Exclusion of other etiologies of meningitis and
- Positive response to anti-tuberculous therapy.
- Probable- M.tuberculosis not demonstrated but features 1, 3 and 4 present.

Cryptococcal meningitis was diagnosed on the basis of CSF findings and positive India ink. Patients having normal CSF findings were diagnosed on the basis of their CT/MRI reports.

Patients having features suggestive of toxoplasmosis, PML, cerebral oedema or cerebral atrophy were diagnosed as such on the basis of their findings.

Methods Of Statistical Analysis

Statistical software Epi info software (3.4.3 version) was used for data analysis.

Coefficient of correlation was calculated to determine correlation between CD4 count and WHO staging.

Ethical Requirements

Patients were informed about the study and a written consent was taken from the patients prior to the procedure. The consent was taken on a specified consent form.

II. Results

In the present study 73 cases (72.27%) belonging to age group 30-49 years were affected, followed by 15-29 years of age group. 81 cases (80.19%) of males were affected, followed by 20 cases (19.80%) of females. 47 cases (46.53%) were on treatment, 35 cases (34.65%) were not on treatment and 19 cases (18.81%) were on interrupted treatment. 81 cases had HIV partners as mode of transmission of virus while 11 cases didn't know the source of infection. Headache was the most common neurological symptom seen in 81 cases (80.19%), followed by seizures in 24 cases (23.76%) and behavior changes in 16 cases (15.84%). 80 cases (79.20%) were of WHO stage 4 and 21 cases (20.79%) of stage 3. 81 cases (80.19%) had CD4 count <200/mm³ and 20 cases had CD4 count between 200-500/mm³. The coefficient of correlation between CD4 count and WHO staging was 0.84.

Table: 1.1 CSF findings in Neuro AIDS patients

CSF finding	No. of patients	Percentage
PROTEIN		
HIGH	64	63.36%
NORMAL	37	36.63%
SUGAR		
LOW	53	52.47%
NORMAL	48	47.52%
CELL COUNT/MM ³		
HIGH	55	54.45%
NORMAL	46	45.54%

DLC		
LYMPHOCYTOSIS	51 OUT OF 55	92.72%
NEUTROPHILIA	04 OUT OF 55	7.27%
AFB		
POSITIVE	01	0.99%
NEGATIVE	100	99%
GRAM STAIN	1 positive	0.99%
INDIA INK		
CRYPTO	14	13.86%
CANDIDA	02	1.98%
Negative	85	83.16%

36 patients (35.64%) showed positive CTs findings and 6 patients (5.94%) showed MRI findings. Final diagnosis on basis of CSF and CT/MRI findings were given as: TBM in 32 cases (31.68%), viral encephalitis in 15 cases (14.85%), cryptococcal meningitis in 14 cases (13.86%). 4 cases of TBM had hydrocephalus. PML was the least common manifestation seen in 1 case (0.99%) only.

III. Discussion

In the present study 80.19% of patients were male and 19.80% females. 72.27% of patients belonged to 30-49yrs of age group, as this age group is sexually more active while 18.81% were between 15-29yrs of age. This is in concordance with the study conducted by Rangnathan et al⁹ in 2004. Male: female ratio was 4:1 in the present study. While Sonkar SK et al¹⁰ reported M:F ratio as 4:1. In India, males are the bread earners and most of them harbour the disease from commercial sex workers. Present study reported 46.53% (47patients) taking treatment, 35.67% patients (35 patients) not on treatment and remaining 18.81% patients taking interrupted treatment. After initiation of ART, opportunistic infections (OI) and other HIV-related events still occur secondary to a delayed recovery of adequate immunity.¹¹

Fig 1.2 Comparison of various Neurological symptoms with different studies:

Symptoms	Sonkar et al ¹⁰ 2012	Bolokadze N et al ¹² 2008	Pal et al ¹³	Present study 2014 (n=101)
Headache	67.4%	91%	-	81.18%
Seizures	14%	15%	33%	23.76%
Behaviour change	-	41% (cognitive dysfunction)	-	15.84%

The difference in the percentage of patients having behaviour change can be due to the sample size taken, as the study conducted by Bolokadze N et al¹² had 388 patients while our study had 101 patients. In the present study 81 cases (80.19%) had CD4 count less than 200/mm³. This correlates with the findings of other studies done by Sharma SK et al¹⁴, Likittanasombut P et al¹⁵ and Sonkar SK et al.¹⁰ They reported 82.6%, 96% and 63.2% patients with less than 200/mm³ CD4 count respectively.

20 cases (19.80%) were reported with CD4 count between 200/mm³ to 500/mm³ in the present study. Patients having CD4 count between 200/mm³ to 500/mm³, 14 patients were of tubercular meningitis (TBM), 2 patients were of pyogenic meningitis (PM), 1 of fungal meningitis (FM) and 3 were undiagnosed. This correlates with the study conducted in Melbourne by Crowe S M et al.¹⁶ They reported that, tuberculosis and oropharyngeal candidiasis develop when CD4 count is in range of 250-500/mm³. When the CD4 count decline to approximately 100/mm³, the common AIDS defining OIs develop, such as toxoplasmosis, cryptococcal meningitis etc. Patients were classified into different HIV stages using WHO staging system. 80 cases (79.20%) in the present study belong to WHO stage 4 while remaining 21 cases (20.79%) belong to stage 3. This was an expected finding as this study was carried on Neuro AIDS patients. An attempt was made to see the correlation between CD4 count and WHO staging which co-related well with coefficient of correlation of 0.84 but 2 cases had CD4 count less than 200/mm³ and which belonged to WHO stage 3. 1 case was diagnosed as TBM and 1 was undiagnosed. Edathodu J et al¹⁷ found 20 patients in stage 3 with CD4 count less than 200/mm³ and coefficient of correlation of -0.65.

Fig 1.3 Comparison of various percentages of neurological manifestations diagnosed on basis of CSF findings and/or CT/MRI with different studies.

Findings	Singh R et al ¹⁸	Sharma SK et al ¹⁴	Sonkar S K et al ¹⁰	S.Chandra et al ¹⁹	Lewy et al ²⁰	Bolokadze et al ¹²	Deshpande et al ²¹	Present study
TB/TBM	14.18%	71.1%	70.0%	34%	0	34%	8%	31.68%
CM	6.01%	3.7%	23.3%	37%	5.03%	15%	17%	13.88%
Pyogenic meningitis	--	--	--	--	--	--	4%	3.9%
Fungal meningitis	--	--	--	--	--	--	--	1.9%
Viral encephalitis	--	--	4.66%	--	--	--	--	14.85%

Toxoplasmosis	0.72%	3.7%		13%	5.66%	22%	20.33%	4.9%
HAD	33.60%	--	9.30%	5%	--	2.40%	1.33%	3.9%
Infarct								1.98%
Hydrocephalus								2.95%
Cerebeller demylination	--	--	4.66%	--	--	---	--	1.98%
Cerebral atrophy								3.9%
Cerebritis	--	--	--	--	--	--	--	3.9%
SOL	1.44	-	6.98%	8%	3.46%	10.7%	21%	2.97%
PML	0.72%	0.7%		1%	0.63%	5%	6.67%	0.99%
Lymphoma		1.5%					5%	--
Undiagnosed	--	--	--	--	--	--	---	5.94%
Total								100%

Attili SVS²² et al has reported TBM in 25.1% patients and CM in 11.0% of cases. 15 cases (14.85%) had viral encephalitis in the present study. It occurs in 15 - 40% cases of HIV, though no systemic studies are available.²³ 6 patients were classified under undiagnosed category as they complained of single episode of seizure, with normal CSF and CT/MRI findings.

All the patients had undergone CSF tap in the present study and CSF abnormalities were seen in 81 patients. 86.13% (87 cases) had clear CSF. 1 patient of TBM had cob web formation and 13 patients had turbid CSF out of which 8 patients were of TBM. Out of 101 cases 64 cases (63.36%) had increase protein while 55 cases (54.45%) had pleocytosis. CSF sugar was low in 53 cases (52.47).

32 cases (31.68%) showed features suggestive of TBM (tubercular meningitis), 7 cases (8.91%) were diagnosed with viral encephalitis by their CSF findings, 1 case had both CSF and CT/MRI findings and remaining 6 cases were diagnosed purely on CT/MRI. 14 cases (13.86%) had cryptococcal meningitis, 4 cases (3.9%) had pyogenic meningitis and 2 cases (1.9%) had fungal meningitis.

Fig: 1.4 CSF comparison of Tuberculous meningitis patients with other studies.

TBM	Thwaites G.E et al ²⁴ 2002	Cecchini D et al ²⁵ 2009	Present study 2014
CSF sugar mg/dl	--	28 (20-42)	29.5 (10-56)
CSF proteins g/L	--	0.77 (0.37-1.45)	0.85 (0.32-1.40)
CSF cells /mm ³	152 (34-430)	47 (6-252)	100 (30-470)

Values are median (range)

Fig: 1.5 CSF comparison of TBM patients with other study

TBM	Katrak et al ¹⁰ 2000	Present study 2014	TBM
CSF Sugar g/l	0.46±0.21	0.31±0.11	CSF Sugar g/l Range
Range	0.25-1.00	0.1-0.56	
CSF Proteins g/l	1.24±0.88	1.1±0.57	CSF Proteins g/l Range
Range	0.45-3.26	0.32-2.68	
CSF cells /mm ³	146±139	147±129	CSF cells /mm ³ Range
Range	36-550	30-470	

Values are mean.

Studies done by Bergemann A et al,²⁶ Bekondi C et al²⁷ and Likkittanasombut P et al¹⁵ reported inflammatory CSF (increased protein, low sugar and pleocytosis in range of 0-2340×10⁹/l) in TBM patients. HIV infected individuals with TBM may therefore have a non inflammatory CSF characteristics.^{25,28} In such cases, diagnosis of TBM cannot be excluded on the basis of normal CSF cell count. A high level of suspicion is necessary.²⁵ In the majority of studies TBM diagnosis is presumptive and based on a combination of clinical, laboratory and radiological findings, emphasising the need for development of a standardized case definition.²⁹

In the present study, CSF cells ranged varied from 30/mm³ to 470/mm³ and CSF protein was markedly low, as seen in above mentioned studies, hence CSF cells and protein of TBM patients may have a varied range, and lower CSF cell count and protein values doesn't rule out TBM.

In the present study one patient stained positive with AFB (acid fast bacilli) as the yield for acid fast bacilli on CSF is low.³⁰

CSF culture for M. tuberculosis was positive in 5 patients (15.6%). This could be due to the fact that CSF protein concentration was less in the present study and absence of neutrophils in remaining cases. High protein CSF concentration and large proportion of CSF neutrophils in CSF (markers of the acute phase), are associated to higher bacteremia, leading to positive cultures.³¹

In the present study 14 cases (13.86%) were diagnosed with Cryptococcal meningitis on the basis of clinical findings and CSF India ink preparation. All the cases of cryptococcal meningitis were positive on India ink.

One patient's CSF was gram positive for diplococci (streptococci pneumonie). The poor yield of Gram stain and culture, could be due the fact that these cases were on antibiotic treatment before hospital admission.²⁷

35 cases (34.64%) showed CT findings and 6 cases (5.9%) had MRI findings (3 cases of encephalitis, 1 case of cerebritis, 1 case of tuberculoma and 1 case of hydrocephalus).

In conclusion, from present study and other studies conducted on CSF, it shows that lymphocytic range varies markedly as well as rate of culture positivity and AFB demonstration on CSF was also low, so high index of suspicion is necessary to confirm or to rule out tuberculosis. More advanced test like PCR should also be done on CSF. Though routine CSF examination is the first line of investigation, it is easy and cost effective method, but more additional tests should also be conducted, where CSF is inconclusive.

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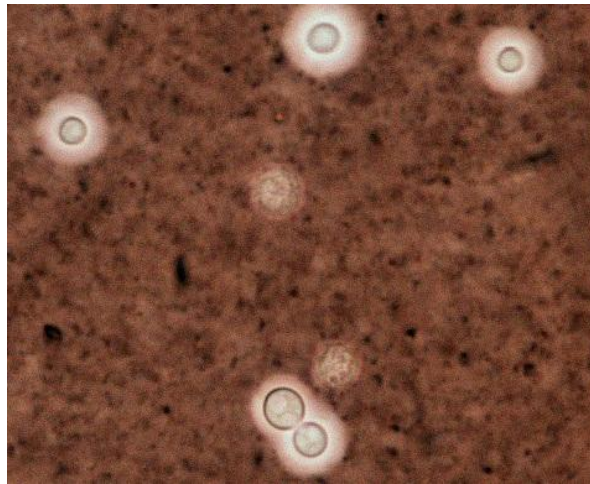


Fig 1 cryptococcus in CSF India ink preparation. (1000x)

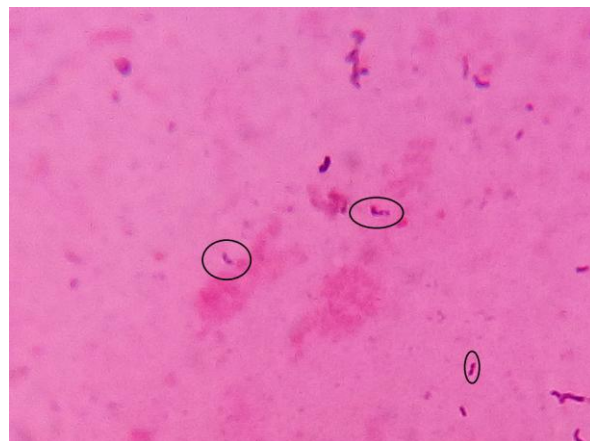


Fig 2 pneumococcus in chain and rods in CSF . Gram stain (1000X)

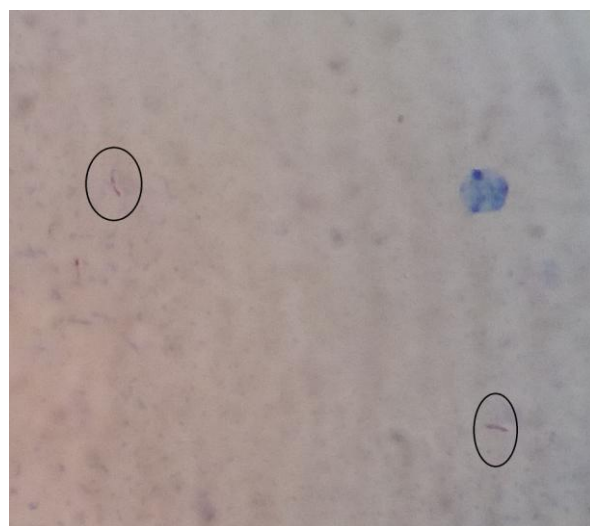


Fig 3: Mycobacterium tuberculosis positive on AFB in CSF. (1000x)