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A Study of Cerebrospinal Fluid Analysis in HIV Positive Individuals

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ABSTRACT

Background: Classical clinical features and laboratory diagnosis are rarely seen in HIV positive patients due to the effect of suppressed immunity and unusual organisms involved. This study aims to emphasize the importance of a timely CSF examination in HIV patients suspected to have CNS disease and correlating it with clinical and radiologic findings which will further help in the management of the disease.

Materials & methods: The present study was a prospective analysis carried out over a period of 2 years where CSF samples from diagnosed HIV positive patients with clinically suspected diseases of the CNS were studied. The CSF samples were analyzed for pathological (Gross examination, Protein, sugar, microscopic examination, ADA levels) and microbiological parameters (special stains and culture).

Results: Out of 215 CSF samples received over a period of 2 years, 90 cases were diagnosed as meningitis on CSF examination. Amongst these, 26 cases (28.88%) were found to be HIV positive. All HIV positive cases had meningitis. Of these 15 (57.7%) were cases of TBM and 11 (42.3%) were cases of Cryptococcal meningitis. Maximum cases were males with a male: female ratio of 3:2. In cryptococcal meningitis, the total protein varied from 53 mg/dL to 174.1mg/dL with a mean value of 88.57 mg/dL, which was lower than that seen in TBM (mean value 185.4mg/dL). The glucose level varied from 12.6 mg/dL to 62.4 mg/dL with a mean level of 38.75 mg/dL as compared to TBM with a mean value of 34mg/dL. The cases of TBM had a CD4 cell count between 54-640 cells/µL whereas the cases of cryptococcal meningitis had lower CD4 counts ie. 12-98 cells / µL. Death was the final outcome in 8 cases of TBM while only 2 cases of cryptococcal meningitis expired and 9 cases were discharged without any neurodeficit.

Conclusion: For proper evaluation of CSF in HIV patients, one should have adequate knowledge about the tests to be performed in various CNS diseases, normal ranges with respect to the patient's age and the test's limitations. Also in some cases where clinical signs and symptoms may be subtle and radiology may not show significant abnormalities, the diagnosis rests solely on the identification of the etiologic agent in the CSF.

KEYWORDS : CSF, HIV, CD4 count, Cryptococcal meningitis, Tuberculous meningitis

INTRODUCTION

CSF analysis was the first ancillary investigation to be introduced into the practice of clinical neurology and for a considerable period of time stood alone as a diagnostic aid to the physician. Different forms of meningitis are associated with HIV depending on the patient's current immune status as per CD4+ T lymphocyte count.[1] Classical clinical features and laboratory diagnosis are rarely seen in these patients due to the effect of suppressed immunity and unusual organisms involved. In the presence of constitutional symptoms, headache and altered mental status, meningitis should be suspected.[1]

The most important indication for CSF analysis in HIV patients is suspected cases of meningitis. This study aims to emphasize the importance of a timely CSF examination in HIV patients suspected to have CNS disease and correlating it with clinical and radiologic findings which will further help in the management of the disease.

MATERIALS & METHODS

The present study was a prospective analysis carried out in a tertiary care hospital over a period of 2 years where CSF samples from diagnosed HIV positive patients with clinically suspected diseases of the CNS were studied. Post mortem CSF samples were excluded from the study.

The CSF samples were analyzed for the following pathological and microbiological parameters:

Pathologic Examination:

1. **Gross examination**: Quantity, colour, turbidity +/-
2. **Protein estimation:** Using 5% trichloroacetic acid (TCA)
3. **Sugar:** Using glucose oxidase – peroxidase (GOD_POD) reagent
4. **Microscopic examination**: Total and differential cell count
5. **CSF ADA levels:** for tuberculous meningitis

Microbiologic examination:

Special stains: Gram's stain, Ziehl Neelsen stain, India-ink, Alcian blue – Periodic acid Schiff (AB – PAS) and Mucicarmine stain.

Culture - Blood agar (BA), chocolate agar (CA) and MacConkey agar, Sabouraud's Dextrose Agar, modified Lowenstein Jensen (LJ) medium with Gruft Mycobacterial supplement. Smear culture and antibiotic sensitivity testing was done for all CSF samples. Also, culture with LJ medium was done in all cases.

India ink was used to demonstrate the capsulated round to oval yeast cells – the capsule standing out as a halo against the dark background. A tap-water control was also made to ensure that the India ink is not contaminated and AB-PAS and Mucicarmine stains were used to demonstrate the polysaccharide capsule of the yeast.

RESULTS:

Out of 215 CSF samples received over a period of 2 years, 90 cases were diagnosed as meningitis on CSF examination. Amongst these, 26 cases (28.88%) were found to be HIV positive. These constituted the sample size. All HIV positive cases had meningitis. Of these 15 (57.7%) were cases of TBM and 11 (42.3%) were cases of Cryptococcal meningitis. Maximum cases were males with a male: female ratio of 3:2.

The common presenting complaints included fever, headache and altered sensorium (Table 1).

Clinical features	TBM (n = 15)	Cryptococcal meningitis(n = 11)
Fever	14 (93.33%)	10 (90.90%)
Headache	15 (100%)	11 (100%)

Clinical features	TBM (n = 15)	Cryptococcal meningitis(n = 11)
Neck rigidity	12 (80%)	8 (72.72%)
Vomiting	5 (33.33%)	3 (27.27%)
Seizures	4 (26.66%)	7 (63.63%)
Altered sensorium	14(93.33%)	10 (90.90%)
Neurodeficit	3 (20%)	1 (9.09%)
Papilloedema	1 (6.66%)	1 (9.09%)

The common radiological features included basal exudates (46.6%) in tuberculous meningitis and leptomeningeal enhancement in cryptococcal meningitis (46.6%). A normal study was obtained in 6 cases. Some cases had more than one radiologic finding, e.g. Space occupying lesion with leptomeningeal enhancement **(Table 2)**.

Table 2: Radiologic Features in HIV positive patients

Radiologic Features	TBM (n = 15)	Cryptococcal Meningitis (n = 11)
Leptomeningeal enhancement	4	7
Basal exudates	7	0
Infarcts	2	0
Hydrocephalus	3	0
REL* / SOL#	2	1
Normal study	2	4

* **REL** – Ring Enhancing Lesion # **SOL** – Space Occupying Lesion

In cryptococcal meningitis, the gross appearance of CSF was slightly hazy in all cases. The supernatant was clear and a deposit was present in all cases. The total protein varied from 53 mg/dL to 174.1mg/dL with a mean value of 88.57 mg/dL, which was lower than that seen in TBM (mean value 185.4mg/dL). The glucose level varied from 12.6 mg/dL to 62.4 mg/dL with a mean level of 38.75 mg/dL as compared to TBM with a mean value of 34mg/dL.

In our study CSF ADA > /= 10IU/L has been used as a cut off for diagnosing TBM.

The cases of TBM had a CD4 cell count between 54-640 cells/µL whereas the cases of cryptococcal meningitis had lower CD4 counts ie. 12-98 cells / µL. Death was the final outcome in 8 cases of TBM while only 2 cases of cryptococcal meninigitis expired and 9 cases were discharged without any neurodeficit **(Table 3)**.

Table 3: CD4 count and final outcome of meningitis in HIV positive patients

Type of Meningitis	CD4 count cells/µL (mean)	Discharge	Discharge with neurodeficit	Death
TBM	54 – 640 cells (207 cells)	6	1	8
Cryptococcal	12 – 98 cells (55 cells)	9	0	2

White, round, moist, convex colonies appeared after incubation on Sabouraud's dextrose agar for 48 hours in all cases of cryptococcal meningitis, the Gram stain of which showed capsulated yeast cells. A positive urease test confirmed the growth as *Cryptococcus neoformans*. Culture with LJ medium showed positivity in 5 cases of TBM.

DISCUSSION:

Although patients with HIV/AIDS are at increased risk of certain types of meningitis like cryptococcal and tuberculous meningitis, they are also more susceptible than the general population to acute bacterial meningitis.[1] Meningitis in HIV positive patients is related to the CD4 cell counts. With early fall in CD4 counts; aseptic meningitis and bacterial meningitis can be seen while tuberculous meningitis and cryptococcal meningitis are seen with marked fall in CD4 cell counts as was seen in our study.[1] We did not encounter any cases of aseptic meningitis and pyogenic meningitis in our study.

As in our study, a review of literature also indicated that the clinical presentation of meningitis in HIV seropositive individuals appears to follow the known pattern of presentation in HIV seronegative individuals. The main presenting feature of cryptococcal meningitis was headache and neck stiffness while patients with TBM came to the hospital with marked mental impairment, compared with that seen with other forms of meningitis. [2,3,4]

Meningeal enhancement on CT scan is more common in HIV infected individuals with TBM.[5] In our study, meningeal enhancement was more common in cryptococcal meningitis as compared to TBM.

The CSF findings in seropositive patients are different – the mean WBC counts in CSF are lower and as many as 16% may have acellular CSF, the mean protein level is 125mg/dL (range of 50 – 200 mg/dL) as many as 43% of patients may have normal CSF protein. Most cases of TBM and cryptococcal meningitis in literature showed lymphocytic predominance but occasional cases showed only neutrophils.[2,4,6,7] In a study by Schutte[8] on TBM, CSF findings showed a lymphocytic predominance, high protein and low glucose values as in our study and were similar in both HIV positive and negative individuals. In a study conducted by Berenguer et al[3] on TBM, analysis of cerebrospinal fluid showed pleocytosis and hypoglycorrhachia but in 43 percent the level of protein in cerebrospinal fluid was normal.

The possible reason for more cases in our study with deranged CSF picture as well as greater positivity of meningeal signs and greater number of cases with leptomeningeal enhancement on radiologic examination may be due to delay by the patients in seeking treatment with presentation after development of a more florid picture of meningitis.

11 cases of fungal meningitis were found in our study and the etiologic agent isolated in all our cases was Cryptococcus neoformans. Of these 9 were known cases of HIV while in 2 cases HIV testing was done after Cryptococcus was isolated in the CSF. Cryptococcus neoformans has been found to be the commonest form of fungal meningitis in literature and its incidence in HIV patients varies from 2.09– 68.6% in various studies.[4] The age distribution of Cryptococcal meningitis varied from 32 to 45 years in our setting which was similar to that found in other studies.[4]

The etiologic agent, Cryptococcus neoformans was isolated in all cases (100%) on India ink examination as well as on culture on Sabouraud's Dextrose Agar while literature states positivity of 70 to 90% for India ink and 80 to 92% for culture.[4] The reason for higher positivity in our study on India ink could be that most of our cases were known cases of HIV with high suspicion for Cryptococcal meningitis and an active search for the organism was made on India ink. AB – PAS and Mucicarmine stains have been used in some other studies to demonstrate the polysaccharide capsule of Cryptococcus neoformans.[9,10]

The latex antigen test (LAT) for polysaccharide antigen, though not performed in our study, has been reported to be highly sensitive and specific in detecting C. *neoformans* infection. However occasional false negatives have been reported and it is strongly recommended that fungal cultures should also be performed. [6]

In all cases of Cryptococcal meningitis the CD4 count was less than 100 cells/µL and in cases where the outcome was death the CD4 count was </= 15 cell/µL which is in accordance with other studies.[1,4]

Mycobacterium tuberculosis yield is better from CSF cultures in HIV infected individuals with TBM.[11,12] In our study, Culture with LJ medium showed positivity in 5 cases of TBM.

In a study by Hakim et al[2], the number of HIV positive patients among cases of meningitis was 90%, the proportion being above 80% in all types of meningitis, with 100% seropositivity among cases of Cryptococcal meningitis. Various studies conducted in different parts of the world, including India, have found the prevalence of cryptococcosis in HIV-reactive patients to range from 2.09% to 68.6% , while in our

study 11(42.3%) cases were of cryptococcal meningitis and 15 (57.6%) were of TBM.[13,14,15] The etiology of meningitis in HIV endemic areas has been significantly altered in favour of Cryptococcal meningitis and TBM.[2]

The CD4 count in TBM cases varied from 54 to 640 cells with a mean of 207cells/µL, as shown in Table 3, which is similar to other studies. CD4+ cell counts less than 200 cells/µL were associated with a poor prognosis.[3,8] In Cryptococcal Meningitis the CD4 count varied from 12 to 98 cells/µL with a mean count of 55 cells/µL and in cases where the outcome was death the CD4 count was </= 15 cell/µL.

In a study by Berenguer et al[3], the mortality was 33 percent in TBM cases with HIV coinfection. In our study, the final outcome was death in 8 cases (53.33%) of TBM and 2 cases (18.18%) of Cryptococcal meningitis.

We have not found any case of concurrent cryptococcal and tuberculous infection. Concurrent cryptococcal infection with tubercular meningitis has been reported in up to 33% of AIDS patients in India.[4]

In a study by Thakur et al[4] on meningitis in HIV positive patients, 46% were found to be positive for *Cryptococcus neoformans* on CSF analysis and 2 patients among the HIV-positive cases were positive for *Mycobacterium tuberculosis* in CSF culture which indicates a high prevalence of Cryptococcal meningitis in HIV positive patients.

Thus, a timely and appropriate analysis of CSF in HIV patients can help the clinician to direct the line of treatment and enhance patient care as well as reduce morbidity and mortality. For proper evaluation of CSF one should have adequate knowledge about the tests to be performed in various CNS diseases especially certain diseases specific to HIV patients, normal ranges with respect to the patient's age and the test's limitations. Also in some cases where clinical signs and symptoms may be subtle and radiology may not show significant abnormalities, the diagnosis rests solely on the identification of the etiologic agent in the CSF.

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