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ANALYSIS OF CSF BY CYTOLOGICAL EVALUATION: AN EARLY AND A COST EFFECTIVE DIAGNOSIS OF MENINGITIS

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ABSTRACT

Introduction: Meningitis is the severe and lethal infection spread worldwide. The most common forms of meningitis are bacterial meningitis caused by Neisseria meningitides and other species. Tuberculous meningitis (TBM) is the most lethal form of Mycobacterium tuberculosis infection, which has a high rate of neurological complications and sequelae. The best method for diagnosis of meningitis is cerebrospinal fluid analysis (CSF). The purpose of present study is to determine the CSF examination.CSF is a clear body fluid, occupying between arachnoid mater and the pia mater. It is formed in the choroid plexus. Infection of the meninges by various pathogens is termed as meningitis. The etiology of meningitis can be bacterial, tubercular and viral. Differentiating bacterial from non-bacterial types is important in deciding the treatment as bacterial meningitis is more prone for life threatening neurological complications and require immediate parenteral antibiotics as compared to nonbacterial antibiotics. The present study is done to evaluate the cytological findings of CSF, along with the clinical history to classify the various types of meningitis. Methods: The present study has been undertaken in the Department of Pathology, Karpaga Vinayaga Hospital, for a period of one year. Lumbar puncture was performed. The direct cell count was done manually using Improved Neubauer counting chamber. Sediment smears were done and stained with Haematoxylin & Eosin, Giemsa stain and special stains like India ink as and when required. Results: The total body fluids evaluated during the study period were 364. CSF accounted for 72 samples. The range of age group varied, between new-borns to 80 years of age. Out of 72 cases of CSF fluid, 60 were normal, 9 were viral, one bacterial and two cases of Cryptococci. The cell count results were correlated with glucose and protein values accordingly. **Conclusion:** This study makes an effort to use the cost effective diagnostic procedure to enable early diagnosis and prompt management, which in turn reduces mortality.

INTRODUCTION

Lumbar puncture is frequently performed in primary care. Properly interpreted tests can make cerebrospinal fluid (CSF) a very important tool in the diagnosis of a variety of diseases. Proper CSF evaluation depends on knowing which tests to order, normal ranges for the patient's age, and the test's limitations. Protein level, opening pressure, and CSF-to-serum glucose ratio vary with age. Xanthochromia is most often caused by the presence of blood, but several other conditions should be considered. The presence of blood can be a reliable predictor of subarachnoid hemorrhage but takes several hours to develop. The three-tube method, commonly used to rule out a central nervous system hemorrhage after a "traumatic tap," is not completely reliable. Red blood cells in CSF caused by a traumatic tap or a subarachnoid hemorrhage artificially increase the white blood cell count and protein level, thereby confounding the diagnosis. Diagnostic uncertainty can be decreased by using accepted corrective formulas. White blood cell differential may be misleading early in the course of meningitis, because more than 10 percent of cases with bacterial infection will have an initial lymphocytic predominance and viral meningitis may initially be dominated by neutrophils. Culture is the gold standard for determining the causative organism in meningitis. However, polymerase chain reaction is much faster and more sensitive in some circumstances. Latex agglutination, with high sensitivity but low specificity, may have a role in managing partially treated meningitis. To prove herpetic, cryptococcal, or tubercular infection, special staining techniques or collection methods may be required. [1]

Frequently perform lumbar puncture by primary care physicians for cerebrospinal fluid (CSF) analysis is an invaluable diagnostic window to the central nervous system (CNS). Routinely performed tests on CSF include protein and glucose levels,cell counts and differential, microscopic examination, and culture.Additional tests such as opening pressure, supernatant color, latex agglutination,and polymerase chain reaction also may be performed. Knowing which tests to order and how to interpret them allows physicians to use CSF as an early and important diagnostic tool in a variety of diseases.

Meningitis is a widespread disease across the world. The cytological Cerebrospinal fluid (CSF) evaluation was developed following the introduction of lumbar puncture in 1891 in Germany. Tumor cells were first reported in the CSF in 1904 and metastatic cancer cells were increasingly identified after 1908. This helped in evaluating cases related to febrile illness and seizure disorder. CSF cytology is now a routine method of investigation of central nervous system (CNS) diseases, worldwide [(2,3)].

CSF is a clear body fluid, occupying between arachnoid mater and the pia mater. It is formed in the choroid plexus. The main function of CSF is to protect the brain and spinal cord from change in pressure; it helps to maintain a stable chemical environment and helps as a media for excretion of waste products of cerebral metabolism.[2,3]

Inflammation of the meninges induced by a variety of pathogens induces anatomical and physiological changes in meninges which lead to loss of integrity of cerebral capillaries, causing leakage of proteins into CSF and migration of polymorphonuclear leucocytes (PMN) into the CSF. Hence an rapid and early diagnosis of meningitis will help in initiation of early treatment and management hence an early recovery for the patients and reduction in the mortality.[3,4]

The etiology of meningitis can be bacterial, viral and tubercular. Differentiating various types of meningitis either bacterial or nonbacterial is key factor in deciding the treatment, as bacterial meningitis is highly risky for life threatening neurological complications and require immediate parenteral antibiotics as compared to non-bacterial meningitis.[5,6]

The present study is done to evaluate the CSF cytological findings , along with the clinical history to classify the various types of meningitis. The present study is done to emphasize the importance of using a very cost effective and early diagnostic approach, helping in management of suspected meningitis cases.

MATERIAL AND METHODS

The present study has been undertaken in the Department of Pathology, Karpaga Vinayaga Hospital, for a period of one year. Relevant clinical information regarding age, sex and accompanying clinical symptoms has been documented. Lumbar puncture was performed by clinicians and further sent in three sterile containers to the collecting unit at Karpaga Vinayaga Hospital. A requisition form with relevant clinical details was sent with the samples. Immune compromised status was also mentioned in order to look for opportunistic infection. It was further processed in pathology, microbiology and biochemistry department respectively. The samples received were immediately processed. Cytological evaluation was performed within 2 hrs. The direct cell count was done manually using Improved Neubauer counting chamber. Cell count for RBC's and WBC was separately done for haemorrhagic tap and corrected WBC count was accordingly reported. The sample was then centrifuged at 3000 rpm for five minutes. Sediment smears were done and stained with Haematoxylin & Eosin, Giemsa stain and special stains like India ink as and when required.

STATISTICAL ANALYSIS

The data was analysed and final results were listed according to frequency and gender. Data was expressed in percentages.

RESULTS

During the study period,the total body fluids evaluated were 364. CSF accounted for 72 samples. The range of age group varied, between new-borns to 80 years of age. Maximum number of cases was in the range of 0-09 years, followed by 30-39 years. Males constituted 40 of 72 cases and females comprised of 32 of 72 cases. Male to female ratio was 1.25:1. In our study out of 72 cases, 61 were clear, 04 were turbid, 03 were xanthochromic, and 04 were hemorrhagic. Microscopically 60 cases showed normal cell count.12 cases showed elevated counts with following pattern of differential count. Lymphocyte predominance was seen in nine cases, neutrophil predominance in a single case and two cases of Cryptococcus which were confirmed by Indian ink preparation. Out of 72 cases of CSF fluid CSF 59 were normal, 10 were viral, one bacterial and two cases of cryptococci. The cell count results were correlated with glucose and protein values accordingly.

DISCUSSION

In pathology of central nervous system, CSF examination is an important diagnostic test . Commonly performed tests on CSF in cytology are physical examination which includes appearance of fluid and supernatant colour. Total leukocyte counts, differential count, and microscopic examination. Biochemical tests like glucose and protein levels are estimated. Cytological evaluation of CSF is a cost effective diagnostic tool for an early diagnosis of meningitis and useful for a better treatment [3,4,5].

Lumbar puncture is a procedure performed to collect CSF for biochemical, microbiological and cytological analysis. This is an important procedure done to diagnosis and differentiation of a variety of infectious and non-infectious neurological conditions [6,7].

Normal CSF is crystal clear (7). The presence of white red blood cells or blood cells, will impart an altered colour to CSF, making it to appear turbid. Xanthochromia is a condition which CSF shows yellowish discoloration. This change in colour is due to lysis of RBC's, thus causing haemoglobin breakdown into methaemoglobin and bilirubin. It is usually seen in patients with newborns and subarachnoid haemorrhage [1]. Newborns will have xanthochromic CSF because of elevated levels of bilirubin and proteins for that age group. Erythrophages are seen before 24 hours after haemorrhage; siderophages containing hemosiderin are encountered 1-2 days after haemorrhage and may persist for 4 weeks. Macrophages haematoid in appear 2 weeks after bleeding. These above mentioned features are signs of previous sub arachnoid haemorrhage [8]. In our study out of 72 cases, 61 were clear, 04 were turbid, 03 were xanthochromic, and 04 were haemorrhagic. A similar study has mentioned that examination of the CSF especially in patients with bacterial meningitis characteristically reveals a cloudy fluid with leucocytosis and neutrophils predominance [9].

The normal CSF contains up to 05 WBC's per cubic mm in adults and 20 WBC's in new born. Increased WBC counts are seen in varying conditions such as, intracerebral bleed, after seizure, inflammatory condition, traumatic tap [10]. In our study, traumatic tap was encountered in 4 cases. In a study of Pinky P et al[3] out of 356 cases, 153 had normal cell count (0-5 cells per cubic mm), while 129 cases had cell count of 5-100 cells per cubic mm and 74 cases had more than 100 cells per cubic mm. in our study we encountered 60 (83.33%) cases with normal WBC count and 12 (16.66%) cases with raised WBC count. In a study conducted by Ali Hassan Abro et al, leucocytosis was noted in 91% of cases with bacterial meningitis and 17% of viral meningitis cases showed mild elevation of white cell count[4]. In a study of Rabab Fouad et al [5] leucocytosis was found in 47.9% of cases with bacterial meningitis, while only in 24.1% of patients with non-bacterial meningitis showed leucocytosis.

The differential count in normal CSF is comprised of approximately 70 percent lymphocytes and 30 percent monocytes. Occasional polymorphonucleocyte can be seen in normal CSF. The differential count alone cannot differentiate bacterial and non-bacterial meningitis. In our study, lymphocyte predominance was seen in nine cases, neutrophil predominance in a single case and two cases of Cryptococci. Cryptococcal meningitis showed lymphocytic pleocytosis in all 2 cases. The study conducted by Pinky et al [3], showed neutrophil predominance in 13 cases of bacterial meningitis and lymphocytic predominance in three cases of fungal meningitis. In a study conducted by Rabab Fouad et al [5], patients with bacterial meningitis had predominantly neutrophilic CSF with neutrophil percentage of more than 50% (69.4%). The patients with non-bacterial meningitis had lymphocytic predominance in 76.5% of cases.

Meningitis can be a lethal disease if left untreated more so in cases of bacterial meningitis. Viral meningitis in immuno competent and aseptic meningitis carries a better prognosis and gets cured within a week or so without any treatment. Clinical differentiation between septic and aseptic meningitis is challenging and rapid diagnosis with treatment reduces the morbidity and mortality associated with the disease [5].

Cryptococcal meningitis is the commonest form of fungal meningitis. It is caused by Cryptococcus neoformans. Cryptococcal infection is commonly occurred in immunocompromised patients with impaired cell mediated immunity. In HIV infection, Cryptococcal infection occurs in advanced stages of disease with CD4+ count less than 50-200 cells/micro L. It occurs in non-HIV patients who are immunodeficient due to cancer, solid organ transplants, diabetes, chemotherauptic drugs, haematological malignancies and very rarely in healthy individuals with no obvious predisposing factors[11].

Clinically, cryptococcal meningitis presents as chronic or subacute meningitis and rarely has a rapid course. Patient present with severe, unbearable headache with or without fever is a characteristic and imortant feature in patients with Cryptococcal meningitis. Fever is seen in only 65% while headache is seen in more than 75% of the patients[12]. Differential diagnosis in suspected case of Cryptococcal meningitis is tuberculosis lymphocytic meningitis and carcinoma. Hence CSF fluid analysis helps in arriving at a correct etiological diagnosis. CSF analysis usually reveals lymphocytic pleocytosis. India ink preparation shows evidence of capsulated Cryptococci. Other methods used are antigen detection as in latex agglutination test and enzyme immunoassay[13].

In our study we encountered two cases of Cryptococcal meningitis, which showed lymphocytic pleocytosis in all two cases and were stained with India ink, to confirm the presence of capsulated Cryptococci. The majority of patients with Cryptoccocal meningitis improve with adequate therapy. Mortality is seen in about 10% of cases. Mortality is more common in HIV positive individuals. Use of anti-retroviral therapy helps to decrease opportunistic infections[13].

CONCLUSION

Meningitis is a major concern of health for few decades. CSF

analysis is a central investigative tool to discriminate Meningitis. In addition, CSF analysis can give significant, quick,rapid and consistent analytic information with positive predictive value and high sensitivity and is very useful in distinguishing Bacterial meningitis and Tuberculous meningitis. It is for developing as well as underdeveloped countries. The major risk factors for severe meningitis are unhygienic conditions, no vaccination; avoidance of timely hospital admissions, lack of medical care. There is need to discover more potent antibiotics for the treatment of severe forms of meningitis Analysis of CSF is an important diagnostic tool of meningitis to differentiate various causes and hence aid in the early diagnosis, treatment and recovery of patient. In immunocompromised patients, an early diagnosis of Cryptococcal meningitis will help in management of patients with in time.

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