

CSF Proteins as Discreminatory Markers of Tubercular and Pyogenic Meningitis

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ABSTRACT

Introduction: Meningitis is still a major cause of illness in many parts of the world. Though substantial improvement has been occurred in the diagnosis of meningitis, conclusive differentiation between tubercular and pyogenic meningitis remains to be an unsolved problem. Patients with meningitis often have severe neurological deficit or die inspite of antibiotic therapy. Thus, improvement in diagnostic test and therapy is required. The objective of the present study was to find a simple biochemical marker for diagnosis of meningitis and differentiation of tubercular and pyogenic meningitis.

Material and Methods: CSF samples were collected from 90 paediatric patients from Nilofer Hospital, Hyderabad, India, from age group of 4 months to 12 years. CSF samples were collected by performing Lumbar Puncture under aseptic conditions and with required precaution. CSF samples were divided into 3 groups where Group 1 included Control that was without CSF inflammation, Group 2 with Tuberculous Meningitis & Group 3 consisting of Pyogenic Meningitis with 30 samples in each group. Electrophoretic analysis of CSF proteins was performed which separated as bands of pre-albumin, albumin, alpha, beta and gamma globulins.

Result: Protein content in CSF was 259 ± 409 mg/dl in tuberculous meningitis, whereas in pyogenic meningitis it was 111 ± 83.94 mg/dl and in control group was 19 ± 13.3 mg/dl. Electrophoretic analysis revealed pre-albumin band to be 2.8 ± 1.2 % in tuberculous meningitis, which was significantly decreased when compared with control and pyogenic meningitis. Albumin band in tuberculous meningitis was 34.8 ± 9.9 %, which was also significantly decreased when compared to control and pyogenic meningitis. Alpha band was 19.7 ± 6.9 % in pyogenic meningitis, but in control and tuberculuous meningitis it was 10.4 ± 2.9 % and 10.3 ± 5.2 % respectively. Beta band was found similar in all the three groups. Gamma band was 33.2 ± 8.08 % in tuberculous meningitis, 13.8 ± 4.55 % in control and 16.7 ± 13.18 % in pyogenic meningitis.

Conclusion: Pre-albumin band was found to be decreased and gamma band was shown to be increased in tuberculous meningitis. Alpha band was increased in pyogenic meningitis. Thus, CSF protein fraction separated and quantitated by native Polyacrylamide slab gel electrophoresis, could be used as markers in differentiation of tubercular and pyogenic meningitis.

Key words: Cerebrospinal fluid (CSF), Tubercular meningitis, Pyogenic meningitis, Polyacrylamide slab gel electrophoresis

INTRODUCTION

In the developing countries, meningitis is common in infants and children. The bacterial meningitis is a serious clinical entity with signs and symptoms that commonly don't allow in distinguishing the diagnosis and the causative agents [1,2]. Tuberculous meningitis is a extra-pulmonary infection and an endemic disease in low socio-economic communities. The diagnosis of tuberculous meningitis gets delayed due to a) inadequate laboratory diagnostic methods b) non-availability of new neuroimaging technique and rapid diagnostic tests c) the clinical presentation is notoriously variable [3]. Early diagnosis of tuberculous meningitis and its differentiation from pyogenic meningitis, specially the partially treated one, might not always be possible in spite of the large battery of investigations. Tuberculous and pyogenic meningitis forms an important group of neurological disease associated with considerable morbidity and mortality [1,4].

The CSF is a dynamic, metabolically active substance that has many important functions [5]. CSF is generated in choroid plexus with the help of specific transporters, carriers and pumps. Normally, CSF is crystal clear fluid composed of 99% water. 80% of CSF protein is serum derived and 20% is produced intrathecally. Protein levels in CSF reflect selective ultra-filtration by epithelial barrier and their secretory ability. Cloudy, purulent, bloody or pigmented CSF has been associated with disease state [6]. CSF is

invaluable diagnostic window to CNS. The procedure of choice to obtain CSF is lumbar puncture. But, the use of lumbar puncture in the diagnosis of central nervous system infections in children has become controversial. Lumbar puncture usage began to decline after concerns were expressed that they might be precipitating brainstem herniation and death in patients. The causal association between lumbar puncture and cerebral herniation remains unproven [7].

The clinical laboratory plays a major role in CSF analysis. The CSF proteins presents with a difficulty in analysis due to their low levels. However, apart from protein and glucose determination in CSF, the clinical utility of other biochemical analyzes in CSF is not often clear. The microbiological analysis provides clue to the etiology. But, the failure to isolate or grow the organism may be due to prior antibiotic use or non bacterial infection and para-meningeal infection. Thus, the diagnosis becomes difficult in the affected patients [3,8]. So, the electrophoretic analysis of CSF proteins has attracted intense clinical interest in diseases of CNS. The CSF electrophoresis may be used to evaluate the clinical disease and can be correlated with similar abnormalities in early stage of the disease process [4]. The aim of the study was to evaluate the utility of CSF protein in diagnosis and differential diagnosis of CNS infections by separation of individual protein fractions by polyacrylamide gel electrophoresis.

MATERIAL AND METHODS

This cross sectional study was performed on CSF samples of 90 paediatric patients with age ranges from 4 months to 12 years, who were admitted in Department of Paediatrics, Nilofar hospital, Hyderabad, India. The study was performed after approval from the institutional ethics committee. The patients were included in the study group after parent or legal guardian provided with written informed consent, if they were unable to read or write then verbal consent was obtained independently. The patients included in the study were diagnosed as tuberculous or pyogenic meningitis based on history, clinical examination and CSF analysis. Patients with metabolic disorder, acute trauma, congenital malformation and bloody CSF samples were not included in the study. The CSF sample was further obtained by lumbar puncture. Lumbar puncture was performed in the interspaces between the lumbar vertebra (L4 and L5). The CSF sample was collected in sterile vial and centrifuged at low speed (1000 rpm) for 5 minutes. CSF sample was grouped as control (Group I), tuberculous meningitis (Group II) and pyogenic meningitis (Group III) with 30 samples in each group. The supernatant was used for the estimation of biochemical parameters as well as for the electrophoresis. CSF protein was estimated by turbidimetric method (Kingsbury et al).

Polyacrylamide slab gel electrophoresis was performed for separating protein molecules in CSF based on the differences in their charge, size and shape at specific pH. The pore size of 5% was used for stacking gel and 7.5% was used for separating gel. The stacking gel was layered on separating gel for preparing the discontinuous gel system. The CSF proteins, were concentrate in small zone of stacking gel. The concentrated CSF proteins separated into bands in spite of its low levels [9,10,11,12]. After the electrophoretic run, the gel was stained for 5-6 hours in freshly prepared Coomassie brilliant blue R-250 stain solution. Stained gels were destained by using destaining solution for about 12 hours by diffusion. The gel background became clear and bands were visible clearly [13]. The photograph of the gel was taken using a digital camera [14]. The images of the electrophoretic run on gel were downloaded on the computer. Using the SCION Corporation software the quantification of the electrophoretic run was performed. The percentage of pre-albumin, albumin, alpha, beta and gamma globulins was calculated.

Parameters	Reference range	Control	Tuberculous Meningitis	Pyogenic Meningitis
Proteins (mg/dl)	0 - 45.6	19 ± 13.3	259 ± 409	111 ± 83.94

[Table/Fig-1]: Mean and Standard Deviation of CSF proteins

Groups	Pre-albumin	Albumin	α Globulin	β Globulin	γ Globulin
Reference range	2.6 – 23.8	27.3 – 58.5	14.6 – 16.2	5.2 – 33.1	3.8 – 23.7
Group I	13.2 ± 5.3	42.98 ± 7.8	10.4 ± 2.9	19.2 ± 6.99	13.8 ± 4.55
Group II	2.8 ± 1.2	34.8 ± 9.9	10.3 ± 5.2	18.7 ± 5.5	33.2 ± 8.08
Group III	10.65 ± 5.5	41.9 ± 9.3	19.7 ± 6.9	17.9 ± 5.3	16.7 ± 13.18

[Table/Fig-2]: Mean ± SD and reference ranges for various CSF protein fractions in paediatric patients. Protein fractions expressed as percentage of total proteins

Groups	Pre-albumin		Albumin		α Globulin		β Globulin		γ Globulin	
	't'	'p'	't'	'p'	't'	'p'	't'	'p'	't'	'p'
I & II	10.48	<0.001*	3.56	<0.001*	0.09	NS	0.31	NS	11.47	<0.001*
I & III	1.83	NS	0.49	NS	6.79	<0.001*	0.81	NS	1.14	NS
II & III	7.7	<0.001*	2.86	<0.01*	5.93	<0.001*	0.57	NS	5.89	<0.001*

[Table/Fig-3]: Mean values of various CSF protein fractions are compared between different groups using student 't' test and 'p' values
*Significant, NS Non significant

RESULTS

Analysis was performed on 90 patients, grouped into 30 patients as control, tuberculous and pyogenic meningitis. The CSF samples were analyzed for protein biochemically then polyacrylamide slab gel electrophoresis, which was used to separate various proteins as bands. Mean and standard deviation was calculated and the reference range was calculated by formula – X (mean) ± 2SD [Table/Fig-1,2 and 3].

DISCUSSION

Meningitis is the most prevalent CNS infection in our country. There is considerable urgency to establish an efficient diagnostics for these patients. Irreversible brain damage may result while waiting for confirming the diagnosis. Delay in diagnosis and treatment are regarded as a major contributing factors to the morbidity and mortality [15]. Biochemical analysis reveals a significant increase in protein concentration in the tuberculous and pyogenic meningitis [4,16]. Higher protein concentration is observed in tuberculous meningitis when it is compared to pyogenic infection due to breach in blood brain barrier and increased local synthesis of gamma globulins [16]. Culture of CSF was the gold standard for confirming the diagnosis of bacterial meningitis. Antibiotic treatment prior to lumbar puncture can decrease the sensitivity of culture. In tuberculous meningitis, required volume of CSF is large preferably 4.0–5.0 ml. Cultures often take upto 6 weeks for positive identification [17]. Mycobacterial antigens have also been detected by enzyme linked immunosorbant assay (ELISA) method. More recently, Lipoarabinomannan (LAM) antigen detection assay has been developed which is superior to other tubercular antigens. But the studies using LAM - ELISA also reported low sensitivity [18,19].

Using Polyacrylamide gel electrophoresis, the normal CSF proteinogram shows various bands ranging from 7 to 14 in number. The CSF protein is separated into pre-albumin, albumin, alpha globulins, beta globulins and gamma globulins bands in cases and control. In the present study, tuberculous meningitis shows decrease level of pre-albumin band as reported by other studies [4,20,21]. Low levels or disappearance of pre-albumin fraction in tuberculous meningitis may have definite diagnostic importance even in the early stage [21]. The pyogenic meningitis group have increased pre-albumin band when compared to tuberculous meningitis. Other studies also reported an increase pre-albumin due to metabolic disturbance in lipoprotein [20]. Albumin is principally produced by liver. Thus, its level in CSF give some integrated information about changes in the permeability of the blood-CSF barrier and CSF turnover [22]. Tuberculous meningitis group have decreased albumin with increase severity. Low level of CSF albumin has been observed in similar studies conducted on CSF analysis of patients with tuberculous meningitis [20,21]. The pyogenic and tuberculous meningitis group have decreased albumin levels when compared to controls, similar to studies by Sundaravalli et al., [16] and Kamath et al., [20]. Alpha globulins levels in tuberculous meningitis and controls are similar, but in pyogenic meningitis it was significantly raised when compared to controls similar to Phadke et al., [4], Sundaravalli et al., [16] and Kamath et al., [20]. In the present study, beta zone showed no significant alteration in any of the group similar

to other studies [21], and also increase in gamma globulins was observed in tuberculous meningitis. Progressive increase of gamma globulin was slightly increased similar to similar to other studies [4,16,20,21]. In pyogenic meningitis, the levels of gamma globulins is slightly increased similar to Sundaravalli et al., [4,16]. In well defined inflammatory CNS disease, identification of immunoglobulin should be evaluated as a diagnostic tool. Its specificity and its appearance as well as disappearance during the course of disease should be established [23].

CONCLUSION

Decreased pre-albumin and albumin fractions and increased gamma fraction are characteristic features of tubercular meningitis. Increased alpha fraction is characteristic of pyogenic meningitis. Thus, the CSF proteins as fractionated by normal polyacrylamide slab gel electrophoresis can be used as markers in differentiating tubercular and pyogenic meningitis.

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