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RESEARCH ARTICLE

Analysis of Cerebrospinal Fluid in Viral Meningitis Patients

***Afshan Zeeshan Wasti, Farah Jabeen, Saba Raza Siddiqui and Rakhshinda Maher**
Department of Biochemistry, Jinnah University for Women, Karachi-Pakistan, 74600.

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Abstract

Aim: Meningitis - an inflammation of the meninges, globally distributed as either sporadic or epidemic forms. Acute meningitis of infectious etiology involves viruses or bacteria making the differential diagnosis very difficult.
Material and Methods: The present study was designed to evaluate the alterations in different biochemical parameters including glucose, protein, C-reactive proteins (CRP), electrolytes (sodium, potassium and chloride) and enzymes (ALT, AST, ALP, CK and LDH) in the CSF and serum samples of the viral meningitis patients (n=20) and compared with control subjects (n=20).
Results: Our study revealed significant ($p<0.0001$) increase in CSF glucose and protein levels in viral meningitis patients as compared to their respective controls. The C-reactive protein was negative in all the cases of viral meningitis, as it is the diagnostic test to differentiate between viral and bacterial meningitis. Significant decrease ($p<0.0001$) was observed in the CSF electrolytes concentration particularly in case of sodium and potassium levels while insignificant decrease was observed in the concentration of chloride. We observed extremely significant ($p<0.0001$) high levels of all the enzymes (ALT, AST, ALP, CK and LDH in the CSF samples of viral meningitis as compared to their respective normal controls.
Conclusion: The present study suggests that the combination of differential analysis of biochemical indices such as CRP, CSF protein, glucose, electrolytes and enzyme profiles is used to establish the more accurate strategy in order to diagnose viral meningitis and also in differentiating it with other types of meningitis.

Introduction

Differentiating viral meningitis from bacterial meningitis will remain a challenging task in order to accurately evaluate the type of meningitis and for therapeutic decision making for acute meningitis. Acute viral (aseptic) meningitis - a clinical syndrome with a predominance of lymphocytes in the cerebro-spinal fluid (CSF), with no common bacterial agents identified in the CSF, is usually self limiting and considered as the main cause of lymphocyte meningitis. While bacterial meningitis is still considered as a common and serious disease. The symptoms and laboratory assays are often similar and overlapping, death is not uncommon and many who survive are left permanently disabled (Almeida SM. et al., 2007; Scheld W.M., 2004).

The identification of etiological agents was possible in only 10% of all the reported cases, due to incomplete diagnostic work-up for viral or aseptic meningitis. For most cases of viral meningitis non-polio enterovirus are responsible (50% to 80%), especially during summer. The Picornaviridae family is further divided within the enterovirus group: like Echovirus, the Polioviruses and the Coxsackieviruses A & B/ 1, 2.

Almeida SM. et al., (2007) have reported that Enterovirus numbers 70 - 71 show a strong neurotropism, associated with meningio-encephalitis, polio-like paralytic syndromes and Guillain Barré Syndrome. However, Coxsackie virus sub-group B is responsible for 60% of cases of meningitis among children less than three years old. About 4% of the

cases of meningitis are due to the viruses of the herpes family, while meningitis caused by HSV-2; HSV-1, 2 and EBV are associated with recurrent lymphocytic meningitis (Baringer J.R.1992).

The clinical symptoms in acute meningitis include fever, malaise, vomiting, and in some cases, petechial rashes. Among younger children's the signs of meningeal irritation are rare including neck stiffness, Kernig's sign, (an infection of the knee when the limb is placed at a certain degree of relative inflection to the trunk), and Brudzinski's sign, (an involuntary inflection of the limb following a head inflection). Moreover, an inability to feed, vomiting, drowsiness, and convulsions was observed in small children's (Thomas K.E. et al., 2002).

The diagnostic criteria conventionally used for aseptic meningitis includes: the Cerebro-spinal fluid (CSF) analysis, Gram stain and culture (Macfarlane D. E. et al., 1985). However, there is no test that is definitive for or against the diagnosis of bacterial meningitis, if the patients show negative results in the CSF gram stain and culture (Ray P. et al., 2007). Nguyen T.H. et al., (2010) have reported that parameters examined in cerebro-spinal fluid (CSF) are less descriptive in children than in adults. The CSF analysis in case of enterovirus meningitis is practically identical to those of bacterial meningitis while other assays, like Gram stain, latex agglutination, and polymerase chain reaction-based assays shows lack in the sensitivity (Shelburne C. et al., 2008; Roos K.L. et al., 2004). Additionally, in many studies the CSF lactate and CSF and serum glucose ratio have been found useful in differentiating between bacterial and viral meningitis and this combination may permit an accurate prediction of the likelihood of viral versus bacterial meningitis (Jacques J. et al., 2003; Freeman H.R. et al., 2004).

The present study was undertaken to evaluate the biochemical changes in the cerebrospinal fluid of viral meningitis patients and the possible role of CRP in designing strategy for more accurate diagnosis and treatment in these patients.

Material and Methods

The present study was based on the biochemical analysis of CSF in viral meningitis patients. The CSF samples were taken from different Hospitals and Institutes all over Karachi-Pakistan. These samples included were already confirmed as viral meningitis cases as negative result was observed on gram staining and/or bacterial culture - the common tools used for the diagnosis of meningitis.

A separate consent form has been filled for each case including the demographic and clinical features as well as the laboratory results of the patients. The CSF analysis was done on the first spinal tap and it included protein, glucose and the Enzyme profile (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) were measured using chemistry analyzer (automated analyzer STAT Lab 300 Plus) while the quantitative determination of C-reactive protein (CRP) was made by a latex agglutination method (CRP-latex Bio-System Kit). The serum electrolytes (Sodium, Potassium and Chloride) were estimated by a Flame photometer (Jenway Clinical PFP7C) for all the patients and controls.

The CSF samples taken as a control group comprises on the patients whose clinical finding and CSF examination exclude the presence of meningitis, they may suffer from high grade fever with electrolyte disturbances etc. We also used serum samples of normal individuals in order to compare our results.

Statistical analysis was performed using standard statistical software (SPSS version 16.0). All data are expressed as mean ± S.D. The data were also tested using student's t-tests; the significance level was set as $p < 0.05$.

Results

The present assessment was made on the confirmed samples of viral meningitis patients with negative results observed on gram staining and/or bacterial culture.

The biochemical profile of cerebrospinal fluid and serum samples of viral meningitis patients and controls is summarized in **Table -1**. Our analysis revealed statistically significant increase in the concentration of glucose in both serum and CSF samples, with marked increase in CSF/Serum glucose ratio in viral meningitis patients as compared to the control group ($p<0.001$). While total protein concentration was also significantly increased in CSF samples of viral meningitis patients but was insignificant in case of serum samples as compared to control group.

The latex agglutination test of C-reactive protein (CRP) depicts negative results in both viral meningitis patients and control groups. However, CRP is routinely used as a diagnostic marker in differentiating viral and bacterial meningitis cases (**Table-1**).

Table-2 shows the electrolyte levels in the CSF of patients with viral meningitis. The following results differ significantly from the control group as the concentration of sodium and potassium were observed to be significantly decreased ($p<0.0001$) with a slight insignificant decrease in the chloride level in the viral meningitis patients.

The results of enzymatic analysis of ALT, AST, ALP, CK and LDH in serum and CSF samples are depicted in **(Table-3).** The enzymatic analysis of the CSF samples revealed that, in viral meningitis patients the levels of all enzymes were observed to be significantly ($p<0.0001$) increased, as compared to the normal controls**.**

Table-1. Biochemical profile of CSF in viral meningitis patients.

CSF-Variables	Normal Range	Controls	Viral Meningitis
CSF-Glucose mg/dl	50-80	70.8±16.2	143±8.139*
Serum-Glucose mg/dl	80-120	98.89±4.281	108±20.52
CSF-/Serum Glucose Ratio	0 - 0.6	0.71	1.32*
Serum-Protein g/dl	5-9	8.519±0.079	9.2±8.88
CSF-Protein g/dl	0.08-1.0	0.76±1.45	2.56±0.095*
CRP (Latex-agglutination) mg/L	6.0	< 6.0 (-ve)	<6.0 (-ve)

The values are the mean±S.D found in CSF from viral meningitis patients (n=20) and control subjects (n=20).
* Unpaired student's t-test ; p <0.0001.

Table-2 CSF Electrolyte profile in viral meningitis patients.

CSF-Variables	Normal Range	Controls	Viral Meningitis
Sodium mEq/L	144-154	140.0±0.907	133.7±2.43*
Potassium mEq/L	2-3.5	4.430±0.104	2.940±0.70*
Chloride mEq/L	90-130	96.40±4.61	95.95±1.75

The values are the mean±S.D found in CSF from viral meningitis patients (n=20) and control subjects (n=20).
* Unpaired student's t-test ; p <0.0001.

Table-3 Enzymatic profile of CSF in viral meningitis patients.

CSF-Variables	Normal Range	Controls	Viral Meningitis
ALT U/L	5-35 (serum) 5-11 (CSF)	50.86±7.88 7.45±1.56	------ 38.74±2.045*
AST U/L	0-35 (serum) 7.5-25 (CSF)	26.3±6.61 9.7±2.45	------ 40.73±2.98*
ALP U/L	38-126 (serum) ≤5 (CSF)	119.82±13.5 3.78±1.98	------ 13.58±5.60*
CK U/L	25-90 (serum) ≤ 18 (CSF)	97.0±8.422 15.2± 2.57	------ 42.0±3.36*
LDH U/L	105-333 (serum) 5-37 (CSF)	15.4±6.8 7.90±3.05	------ 32.8±16.6*

The values are the mean±S.D found in CSF from viral meningitis patients (n=20) and control subjects (n=20).
* Unpaired student's t-test ; p <0.0001.
----- data not available.

Discussion

In order to differentiate aseptic meningitis to the bacterial meningitis, numbers of studies have shown the effectiveness of rapid and definite tests using CSF variables and markers of peripheral blood for various common and uncommon laboratory measurements (16-18). The present study focuses on identifying different biochemical components of CSF and serum samples in patients with viral meningitis which is more common than bacterial form, but generally less serious although it can be very debilitating.

Marked increase was observed in CSF protein levels (236.8%), consistent with the findings of other studies suggesting 50-100 mg/dl protein concentration in CSF samples of viral meningitis. According to Rot Bart (1997), although the examination of total CSF protein has been found useful in the diagnosis of some neurological disorders such as meningitis, it could not help to any great extent in the diagnosis or differential diagnosis of other diseased conditions. However this increase in protein level is due to the increased membrane permeability may lead to increase CSF enzymes proportionately, helping in the differential diagnosis of meningitis (Almeida SM. et al., 2007).

The glucose level in meningitis may be altered due to the changes in metabolizing glucose by the cells, white blood cells or bacteria due to inflammation. Ejrances et al., 2005 have reported the concentration of glucose might normal with elevated protein level in cases of meningitis. On the contrary, our results suggest a marked increase in the concentration of glucose in the CSF samples of viral meningitis i.e. 101.9%. Additionally, in confirming the diagnosis of bacterial meningitis Jadali F (2007) has reported the negative correlation between CSF glucose and serum CRP revealed the important role of reduced CSF-glucose levels. Ordinarily, a low CSF glucose concentration strongly suggests bacterial meningitis, although Sormunen et al., (1999) found this situation in only one third of bacterial meningitis patients.

Marked significant decrease ($p<0.0001$) in electrolyte concentration was observed in the CSF samples of viral meningitis. The changes in electrolyte levels play a significant role in the diagnosis of viral/ bacterial meningitis as, the inflammation of meninges may cause damage to the blood brain barriers which leads to alter brain homeostasis. Moreover, the patients with fatal outcome of bacterial meningitis showed significantly higher CSF-acidosis and K+ level with lower levels of bicarbonate in CSF (Wiczkowski A et al., 1995).

Our study revealed a significant increase in the CSF enzyme activities (ALT, AST, ALP, CK and LDH) in viral meningitis patients as compared to controls. Many research reports suggest that the prognosis for meningitis patients could not establish on the basis of enzymatic activity alone, but depends on several factors. Sharma N et al., (2006)

have reported that the enzyme activity in CSF was appreciably less than serum in normal subjects as well as in majority of disorders of central nervous system. However different enzyme activities such as LDH, GGT (gamma glutamyltransferase) and CK are elevated in CSF samples of various neurological conditions including infections. The increase in the CSF levels reflects the extent of brain injury as observed in cases of meningitis, may be due to the destruction of blood-brain barriers.

Furthermore, the rise of enzymatic activity in serum cannot be explained on the basis of disturbed BBB, as the enzymatic activity is normally very low in the CSF as compared to serum. It was also observed that during the first few days of acute inflammation of the meninges, the pathological process is at its peak while in treated patients, there is a decrease in the vascularity of the meninges and the enzymatic activity declines. However, the disease was fatal or drug therapy could not control the pathological process in case of increased activity of enzymes on subsequent estimations illustrating disruption of BBB stayed higher (Lampl Y et al., 1990) (21).

Sarojini BL et al., (1991) have reported that the studies on CSF-enzymes such as lactate dehydrogenase, CK, Isocitrate dehydrogenase and transaminases (ALT, AST) plays a critical role in the differential diagnosis of meningitis. The level of ALT, AST and CK was increased but ALP was normal except in two patients having a significantly high level of ALP may be due to the drug treatment. Various other studies have also suggested a rise in serum and CSF AST levels and attributed it to necrosis or anoxic injury to brain tissue (22-24). Furthermore, Sirkis I (1982) has reported that the activity of the CSF Aminotransferases rises during the acute period of meningitis, but higher in meningococcal and tuberculous meningitis than in the viral one however no substantial change was observed in blood serum enzymes (26).

The CRP is an acute phase reactant elevated in case of inflammation. Many research studies strongly suggested CSF-CRP measurements as a reliable test to discriminate viral and bacterial meningitis; along with routine clinical applications (Scheld WM 2004) and are found to be a more sensitive test than total protein and glucose. Presence of CRP is also helpful in differentiating bacterial meningitis from other neurological disorders. Varying values for positivity of C-reactive proteins in CSF samples of viral meningitis were due to various factors (e.g. age of patient, duration and severity of disease, specimen handling, etc., which might explain the discrepancy in results of the CSF-CRP test (Singh U.K. 1994; John, 1990). The C–reactive protein remains negative in all the CSF samples observed in our study further confirm the presence of viral meningitis. In addition, the values of all the parameters in serum of healthy individuals show normal.

Conclusion

The prompt and accurate diagnosis of Meningitis - a life threatening epidemiological problem, is considered as dire consequences especially in developing countries. The present study was designed to evaluate the biochemical changes in the CSF samples, in order to differentiate viral and bacterial meningitis, as laboratory investigations play a major role in early diagnosis of this disease. It is important to understand the utilization of inexpensive, more sensitive and specific rapid tests as the alterations were observed in all the parameters such as protein, glucose, CRP, electrolytes and enzyme profiles in viral meningitis patients. Moreover, on the performance of CRP (C - reactive protein) test, no clumping was observed, which shows negative result, but it may show the vast variation in different CSF constituents in case of viral meningitis. This study may play an important role in the diagnosis and more accurate treatment for the patients suffering from viral meningitis.

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