

GLUCOSE STATUS IN CEREBROSPINAL FLUID (CSF) IN DIFFERENT MENINGITIC CHILDREN IN A HOSPITAL IN CHITTAGONG, BANGLADESH.

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ABSTRACT

Meningitis is referred to as an inflammatory process of the leptomeninges and cerebrospinal fluid (CSF) within the sub-arachnoid space of the brain. We have investigated glucose status in CSF in different types of meningitis together with detailed medical history in children. In addition, we have also carried out the detailed cytological and microbiological examinations. A total of 40 subjects were investigated. We observed that the glucose level was significantly decreased (<20 mg/dl) in 65%, moderately decreased (20-40 mg/dl) in 20% and mildly decreased (40-50mg/dl) in 15% of the patients in our study. Patients with Pyogenic meningitis had tremendously reduced glucose level (9.0 mg/dl) in their CSF whereas in viral meningitis the CSF glucose level is highly variable (10 to 65 mg/dl). Furthermore, 5 (12.5%) patients showed high lymphocyte counts and 34 (85%) patients showed high neutrophil counts. Interestingly, in Pyogenic meningitis, the neutrophil count was very high compared to that in viral meningitis. The present study clearly demonstrates that biochemical parameters such as glucose level in CSF might be a potential tool for detecting meningitis and as well as differentiation of the different types of meningitis.

Key words: Meningitis, Cerebrospinal fluid, Glucose, Pyogenic meningitis.

INTRODUCTION

Cerebrospinal fluid (CSF) is a clear, colorless watery fluid formed by the choroid plexus of the lateral ventricle and the third ventricle of the brain. Based on physical appearance of the CSF, the meningitis could be differentiated

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into pyogenic (cloudy or hazy appearance), haemorrhagic (red/bloody appearance) and yellow color (xanthochromia) (Khaleque 1963). If the CSF is brown, orange or yellow, it may be a sign of increased CSF protein or previous bleeding (Arevalo *et al.* 1989) and could be associated with pyogenic meningitis and or tuberculous meningitis (Conly *et al.* 1983). Meningitis usually results from infection by microorganisms (Fortún *et al.* 1994). A critical event in the pathogenesis of bacterial meningitis is the inflammatory reaction induced by the invading bacteria. Many of the neurologic manifestations and complications of bacterial meningitis result from the host immune response to the invading pathogen rather than from direct bacteria-induced tissue injury (Lehnardt *et al.* 2007). As a result, neurologic injury can progress even after the CSF has been sterilized by antibiotic therapy (Tsubota *et al.* 2010). Viruses that can cause meningitis include enteroviruses, herpes simplex virus type 2, varicella zoster virus, mumps virus, HIV, and LCMV (Logan & MacMahon 2008). Mild cases of viral meningitis can be treated at home with conservative measures such as fluid, bed rest and analgesics (Meningitis and Encephalitis Fact Sheet 2007). The diagnosis of meningitis is made by patients feature of meningism such as neck rigidity, Kernig's sign and Brudzinski's sign (Strachan *et al.* 2008). Diagnosis is also made by biochemical analysis of CSF and microbiological identification of bacteria in case of bacterial meningitis; either by bacterial culture or broad range PCR. However, in many cases in our country, microorganisms could not be isolated because of improper use of broad spectrum antibiotic therapy before the diagnosis is established. So biochemical analysis like CSF glucose, CSF protein might be important parameter to exclude the viral meningitis from bacterial meningitis. Low CSF glucose level usually is associated with bacterial infection both pyogenic and tuberculous. However, glucose level remains normal in case of viral meningitis (Abro *et al.* 2008). So, in this study, we have investigated glucose status in different type of meningitis.

METHODS AND MATERIALS

Patient selection

The study was conducted in the Pathology & Microbiology laboratory, Chattagram Maa Shishu-O-General Hospital, Agrabad, Chittagong, Bangladesh during the period of November 2008 to June 2009. Forty subjects were included in this study. Informed parental consent was taken before enrolling the children into the study. The study subjects comprised of the following four groups: pyogenic-27 subjects with pyogenic meningitis, viral-5 subjects with viral

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meningitis, tubercular-2 subjects with tubercular meningitis and normal-6 subjects were normal, healthy children. All subjects were enrolled everyday between 9:00 a.m. and 2:00 p.m. from the pediatric unit who were admitted into this hospital mainly with the complaints of fever. In all cases, the suspected meningitis subjects were between 0 and 12 years old. A questionnaire was developed to obtain relevant information of demographic and socio-economic data. The questionnaire also included anthropometric data, birth history, immunization history, past medical history and clinical information.

CSF Collection

CSF was collected from the selected patients by the process of lumbar puncture (usually in the space between third and fourth lumbar vertebra) done in the hospital ward with a sterile lumbar puncture needle and stilette with 3 sterile glass vials (one for biochemical, one for cytological and one for microbiological study). 10 ml fluid was collected in each vial, which were sent to laboratory after proper labeling.

Estimation of CSF Glucose

Estimation of glucose level was done by Glucose oxidase method. Freshly collected CSFs were first centrifuged to remove blood cells and particles. Three test tubes were taken and marked them as blank, sample and standard. Ten μ l of sample was taken in sample test tube, 10 μ l standard in standard test tube and 10 μ l purified water was taken in blank test tube. After adding 1000 μ l reagent in all test tubes, it was mixed thoroughly and kept at room temperature for 10 minutes. Absorbance of the sample and standard were measured at 546 nm after adjusting the blank as zero.

Calculation

$\frac{\Delta \text{ Abs. of sample}}{\Delta \text{ Abs. of standard}} \times \text{standard conc.} = \text{Glucose conc. (mg/dl)}$ (Bablok *et al.* 1988).

$\Delta \text{ Abs. of standard}$

Microbiological examination

With all aseptic measures, an aliquot of the CSF was plated on LB agar (Lysogeny Broth) plate and incubated at 37°C for overnight. The presence or absence of organism was detected based on appearance of colonies and colony morphologies.

Cell counting in the CSF

Neutrophil and lymphocyte counts were done by spreading a drop of CSF on a microscope slide. The slide was stained with a Giemsa stain and examined under a microscope at 100X. The neutrophils and lymphocytes were identified based on the shape and appearance of the nucleus and the presence and color of the granules. The neutrophils had granules and polymorphic nucleus. On the other hand lymphocytes had no granules and no polymorphic nucleus, but only a single nucleus.

RESULTS AND DISCUSSIONS

The analysis of the cerebrospinal fluid (CSF) changes in acute meningitis could be good means of discriminating bacterial and viral meningitis in children (Abro *et al.* 2008). The meningitis patients admitted to Chattagram Maa Shishu-O-General Hospital for Infectious Diseases in Chittagong from November 2008-June 2009, with the sign and symptoms of meningitis such as headache, nausea, vomiting, fever, restlessness, irritability, neck pain, poor feeding, neck rigidity, Kernig's sign, Brudzinski's sign, Bulged Fontanel (Table 1 and 2) were included in this study. The study was designed based on the detailed background data of the patients (previous records of infectious diseases/illness, vaccination, socio-economic status) and detailed clinical, immunological and biochemical examinations of their cerebrospinal fluid. The risk factors and side effects were determined by different parameters such as socio-economic condition of the patients attending at Chattagram Maa Shishu-O-General Hospital, Chittagong, Bangladesh.

TABLE 1: SYMPTOMS OF THE PATIENTS IN THIS STUDY.

No. of patients	Symptom	Present (%)	Absent (%)
40	Headache	37.5	62.5
	Vomiting	80	20
	Blurring of vision	85	15
	Convulsion	65	35

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TABLE 2: NERVOUS SYSTEM EXAMINATIONS OF THE PATIENTS INCLUDED IN THIS STUDY.

No. of patients	Neck rigidity	Kernig's sign	Brudzinski's sign	Bulged Fontanel
40	30(75%)	22(55%)	24(60%)	34(85%)

In terms of socio-economic status, the distribution of the patients were upper class (15%), middle class (30%) and lower class (55%), indicating that though the disease can affect peoples regardless of their socio-economic status, lower economic class peoples are more susceptible to meningitis (data not shown). Moreover, most of the patients that exhibited the sign and symptoms of meningitis had the following symptoms: vomiting (80%), headache (37.5%), convulsion (65%) (Table 1). We also observed that meningitis could occur even in those who were vaccinated (Data not shown).

The microbiological studies revealed the presence of Diplococci and Gram (-ve) Bacilli in 6 (15%) and 2 (5%) patients, while no microbial pathogens were detected in the rest 32 (80%) patients (Fig: 3). The absence of any microbial pathogens in the rest 32 patients CSF could be due to the fact that from the very onset of the disease, those patients were treated with antibiotics. But the changes of physiological appearance in their CSF were suggestive of bacterial meningitis (Table 3). These observations greatly deviated from a previous observation. However, it showed some similarities (Hussein and Shafran 2000).

Differential diagnosis between viral and bacterial meningitis is very difficult. Most of the patients of both bacterial and viral meningitis shared common features of meningism; Neck rigidity 30 (75%), Kernig's sign 22 (55%), Brudzinski sign 24 (60%), Bulged Fontanel 34 (85%). Bulged Fontanel was present in 85% patients (Table 2), which is clearly in line with a previous study (Abro *et al.* 2008).

It is well reported that all forms of pyogenic meningitis are frequently associated with leucocytosis and a raised ESR (erythrocyte sedimentation rate) and viral meningitis is associated with high lymphocyte count, though in a few cases lymphocyte count were found normal. In our study, we observed high lymphocyte count in 5 (13%) patients and high polymorphs count in 32 (84%) patients (Fig: 2), which supported the results of Negrini (Negrini *et al.* 2000). They showed that the CSF leukocyte count was higher with predominant

polymorphs (95%) in bacterial than viral (7%) cases. Most of the patients with aseptic meningitis had neutrophils accounted for >50% of CSF leukocytes (Negrini *et al.* 2000).

TABLE 3: PHYSICAL APPEARANCE OF CSF COLLECTED PATIENTS INCLUDED IN THIS STUDY.

No. of patients	Hazy	Clear	Straw
40	24	6	10

TABLE 4: EFFECT OF CSF GLUCOSE LEVEL IRRESPECTIVE OF MICROORGANISM PRESENT AND ABSENT.

No. of patients	Micro-organisms	Glucose Level (Mean±SD)	P*
40	Present (8)	11.75±2.52 (mg/dl)	0.007
	Absent (32)	24.54±12.59 (mg/dl)	

*Student T test was done for the test of significance.

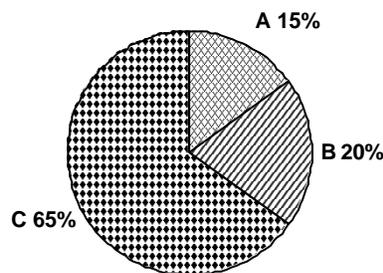


FIGURE 1: GLUCOSE LEVELS (A) MILDLY DECREASED (40-50 mg/dl) (B) MODERATELY DECREASED (20-40mg/dl) & (C) MASSIVELY DECREASED (<20mg/dl).

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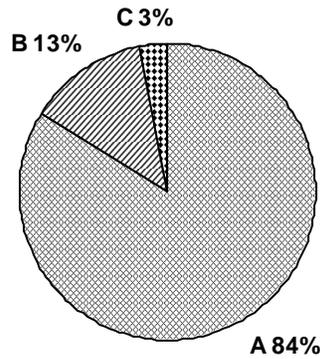


FIGURE 2: NEUTROPHILS, LYMPHOCYTES AND OTHERS (A) 84% OF NEUTROPHIL (B) 13% OF LYMPHOCYTES & (C) OTHERS.

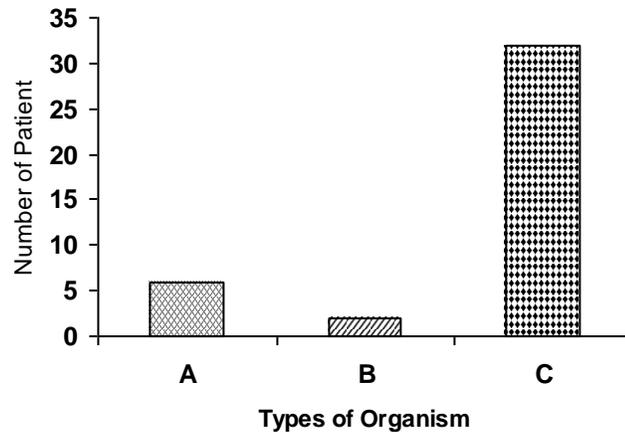


FIGURE 3: NUMBER AND TYPES OF ORGANISMS. (A) PRESENCE OF GRAM+(VE) BACTERIA (B) PRESENCE OF GRAM-(VE) BACTERIA AND (C) ORGANISM NOT FOUND.

The normal CSF glucose level of 45-85 mg/dl was reported (Panda and Dash 2002). In our study, we found that glucose content in CSF was significantly decreased in 26 (65%) while moderately and mildly decreased in 8 (20%) and 6 (15%) of the patients, respectively (Fig: 1). More specifically, glucose level of 11.75 ± 2.52 mg/dl (Mean \pm SD) was observed in the bacterial meningitic group which was significantly ($P=0.007$) different with glucose level of 24.54 ± 12.59 mg/dl (Mean \pm SD) in the nonbacterial meningitic group (Table 4). Similar observations have also been reported by other (Zeni *et al.* 1994 and Bender *et al.* 2010). These observations thus clearly indicate that glucose content in the CSF could be a good parameter for differentiating the bacterial meningitis and organism absent meningitis.

In this study, complete medical history of the patients, microbiological/cytological and detailed biochemical examination of the CSF of meningitis patients helped in a more accurate discrimination of different meningitis in children. In most cases in our country, meningitis suspected subjects bearing the common symptoms of meningitis are treated without considering the different types. Our result clearly demonstrated that the glucose status of the CSF can be an indication of the types of the different meningitis which ultimately could facilitate the disease management more precisely and could avoid unnecessary administration of antibiotics.

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