

Cerebrospinal fluid cholinesterase activity in children with meningitis

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Received 29.9.2010
Accepted 22.11.2010

ABSTRACT

Objective: To assess the changes of cerebrospinal fluid (CSF) acetyl cholinesterase (AChE) activity in children with acute bacterial meningitis in comparison to healthy age and sex matched controls.

Patients and methods: Out of 41 cases admitted to Alkhansaa pediatric and delivery hospital (Mosul city) between January 2010 and July 2010, 28 cases proved to be a case of *Haemophilus influenzae* meningitis, 13 child proved lack from any type of meningitis with age and sex matched subjects taken as a control group. Initially from all of the studied children (patients and controls) about 5 ml CSF samples were taken and assayed for appearance, leucocytes, red blood cells, sugar, protein and AChE activities by spectrophotometric method.

Results: There were a significant increase in CSF WBCs count, RBCs count, protein levels, AChE activity with a decrease in CSF glucose level for children with acute bacterial meningitis in comparison to the control group.

Conclusion: There is an important value for measurement of CSF AChE activity for confidential diagnosis of children with acute bacterial meningitis specially for those with equivocal decision by using the traditional parameters.

Keywords: Bacterial meningitis, CSF AChE.

الخلاصة

أهداف البحث: لتحديد أهمية قياس مستوى التغيير لنشاط خميرة الكولين استراز في سائل النخاع الشوكي للمرضى المصابين بالتهاب السحايا البكتيري الحاد من الاطفال وبالمقارنة مع مجموعة ضبط من اصحاء ذوي اجناس و اعمار مقارنة لمجموعة المرضى.

المرضى وطرق العمل: من أصل 41 مريضا ادخلوا الى مستشفى الخنساء للأطفال و التوليد في مدينة الموصل للفترة ما بين كانون الثاني 2010 و تموز 2010 ، كان هناك 28 حالة شخصت على انها التهاب سحايا بكتيري بجرثومة *Haemophilus influenzae* . كذلك انتخب 13 طفلا من أعمار و أجناس مقارنة من الاصحاء اتخذت كمجموعة ضبط. في البداية تم سحب 5 مل من سائل النخاع الشوكي من المجموعتين (مجموعة المرضى والضبط) وتم معاينة النماذج و فحص كريات الدم البيضاء، كريات الدم الحمر، مستوى السكر، مستوى البروتين و قياس مستوى نشاط خميرة الكولين استراز في سائل النخاع الشوكي باستخدام طريقة امتصاص الطيف الضوئي.

النتائج: هنالك زيادة معنوية في كريات الدم البيضاء، كريات الدم الحمر، مستوى البروتين و مستوى نشاط خميرة الكولين استراز في سائل النخاع الشوكي مع نقص معنوي في مستوى السكر للأطفال الذين لديهم التهاب السحايا الحاد بالمقارنة مع مجموعة الضبط.

الاستنتاج: كان هنالك فائدة مهمة من قياس مستوى نشاط خميرة الكولين استراز في سائل النخاع الشوكي للتأكد من صحة تشخيص التهاب السحايا البكتيري الحاد عند الأطفال الذين لم يكتمل تشخيصهم للمرض باستخدام طرق الكشف الاعتيادية .

Meningitis is an inflammation of the leptomeninges and underlying subarachnoid cerebrospinal fluid (CSF). Bacterial meningitis in infants and children mostly can caused by *H influenzae* (48%), *S pneumoniae* (13%), and *N meningitides*¹. Despite

increasing use of the *Haemophilus influenzae* and pneumococcal vaccines, acute bacterial meningitis is a serious cause of mortality and morbidity worldwide. Evaluation of patients with meningitis is complicated by the fact that clinical findings do not reliably differentiate bacterial meningitis from

other illnesses such as aseptic (viral) meningitis. This is particularly true in young children and infants, making diagnosis in the pediatric age group especially problematic. There is a prevalent myth that CSF, white cell counts and other surrogate markers are reliable indicators of bacterial versus viral infections. This is a dangerous misconception²⁻⁴.

A reliable means of distinguishing between bacterial and viral central nervous system (CNS) infections would reduce the risk and expense of unnecessary antibiotic therapy and hospitalization in aseptic meningitis patients⁵.

The most important diagnostic study for patients with possible meningitis is lumbar puncture with CSF analysis. The gold standard for diagnosing bacterial meningitis is the CSF culture, despite its limited value in patients who have been partially treated. However, physicians must make treatment decisions before culture results are available, and they depend on CSF findings such as cell count, glucose, protein, and Gram's stain to help them do so^{6,7}.

Other CSF studies have been advocated as a means of distinguishing bacterial from aseptic meningitis. These include CSF leukocyte aggregation,⁸ CSF lactate⁹ and CSF cytokine levels, among others. Many authors have studied these tests, but have not conclusively demonstrated their utility. Bacterial antigen studies, more widely used, also have limited utility during the initial patient encounter. To date, no single diagnostic model has been validated in an adequate sample of patients as anything more than an adjunct to physician judgment. The aim of the study is to determine the changes of CSF cholinesterase activity in children with acute bacterial meningitis in

comparison to healthy age and sex matched controls.

Patients and methods

This study was conducted from January to July 2010. Children with positive signs of meningitis were admitted to Alkhansaa pediatric and maternity hospital (Mosul city) and immediately a CSF samples were taken. Later positively proved cases of bacterial meningitis (from CSF culture) were selected and included in this study as the case group, whereas the negatively proved cases represented as the control group.

Out of 41 cases admitted to hospital, 28 cases proved to be bacterial meningitis (all due to *Haemophilus influenzae*). They were 11 females with 17 males with a mean \pm SD age 3.25 ± 2.08 years (ranged between .5 and 8 years). Also included 13 apparently healthy children as a control group. They were 5 females and 8 males with a mean \pm SD age 3.58 ± 2.53 years (ranged between .6 and 8.5 years).

Initially around 5ml CSF samples were taken from patients and control, and assay of appearance, WBC count, RBC count, glucose concentrations were measured by enzymatic colorimetric method which was available as a kit purchased from Biomaghreb¹⁰, protein concentrations were measured by bromocresol green (BCG) method which was available as a kit purchased from Randox Laboratories Ltd^{11,12}, and ChE activities were measured by spectrophotometric Ellman et al. method, after centrifugation of the CSF sample at 3000 rpm for 5 min¹³.

Statistical analysis

All values were quoted as mean \pm SD and a P-value <0.05 was considered to be statistically significant. Unpaired t-test was used to compare results of

patients suffer from bacterial meningitis, with the results of the controls.

Results

There were a highly significant increase in CSF WBCs count, CSF RBCs count, CSF protein levels, CSF AChE activity with a decrease in CSF glucose level for children with acute bacterial meningitis in comparison to the control group (Table 1).

The specific activity indicates how much of the total protein is to be isolated; depending on the following equation¹⁴.

$$\frac{\text{enzyme concentration (unit/mL)}}{\text{protein concentration } \left(\frac{\text{mg}}{\text{mL}}\right)} = \text{specific activity (unit/mg)}$$

Therefore, the AChE specific activity value of the control group equal to 21.066 nanomol min⁻¹mg⁻¹, while that of the meningitis group equal to 15.418 nanomol min⁻¹mg⁻¹. The present study revealed that despite the increase in the CSF protein and AChE activity among patients group versus the control group as shown in Figure 1 and Figure 2, there is a decrease in AChE specific activity in patients group versus control group as shown in Figure 3

Table 1. comparison of CSF WBCs count, RBCs count, protein level, AChE activity and glucose levels between cases of acute bacterial meningitis and the controls.

Parameters	Controls (n=13)	Cases before therapy (n=28)
CSF WBCs count	17±2.76 leukocyte/μL	237±43.76 leukocyte/μL*
CSF RBCs count	.08±.013 RBC/ μL	3.42±.55 RBC/ μL*
CSF protein	390.2±71.2 mg/L	775.38±131.86 mg/L*
CSF AChE	8.22±1.72 nanomol min ⁻¹ ml ⁻¹	11.955±2.62 nanomol min ⁻¹ ml ⁻¹ *
CSF glucose	4.58±.68 mmol/L	2.87±.49 mmol/L*

*p<0.001

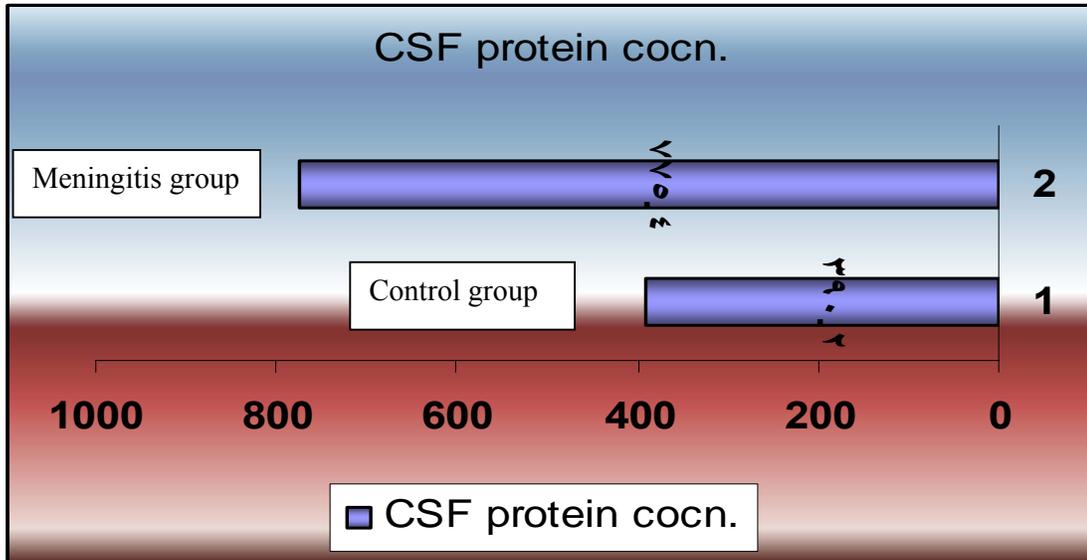


Figure 1. Distribution of CSF protein concentrations among control subjects versus patients group

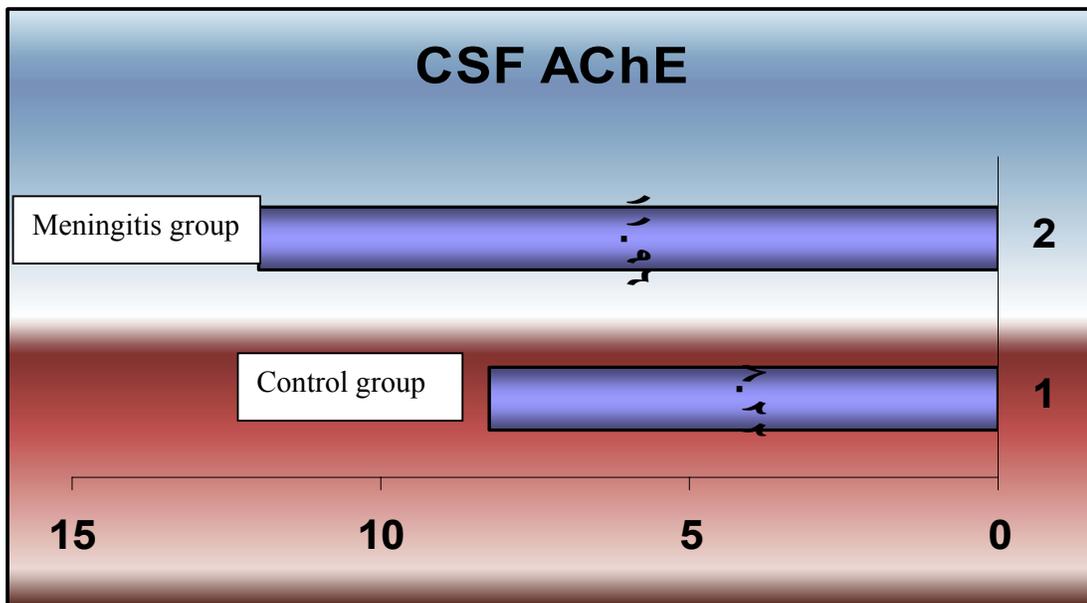


Figure 2. Distribution of CSF AChE activity among control subjects versus patients group

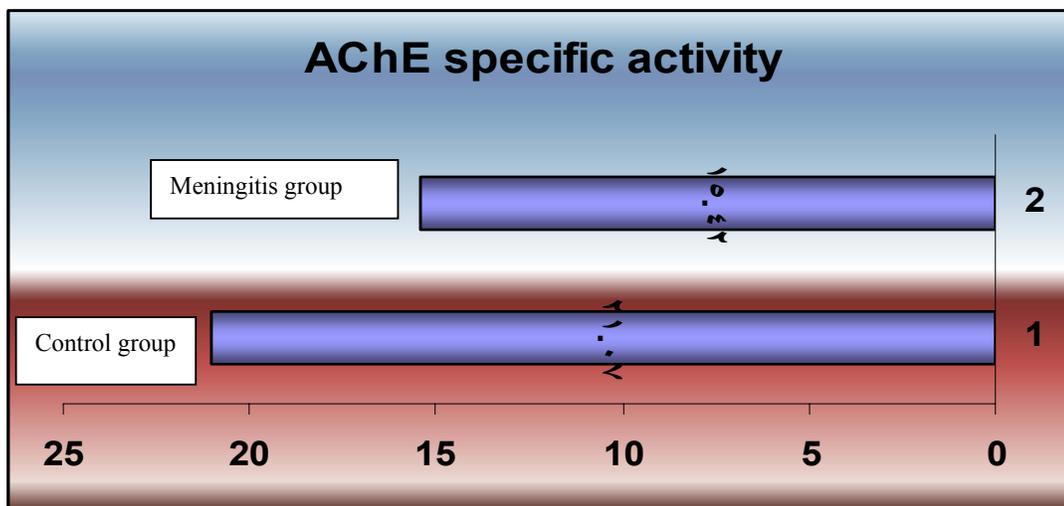


Figure 3. Distribution of CSF AChE specific activity among control subjects versus patients group

Discussion

Distinguishing acute, subacute, and chronic meningitis helps identify the pathogen. Approximately 25% of patients with bacterial meningitis present acutely, well within 24 hours of onset of symptoms. Other patients with subacute bacterial meningitis and most patients with viral meningitis present with neurologic symptoms developing over 1-7 days. Chronic symptoms lasting longer than 1 week suggest meningitis caused by some viruses as well as tuberculosis, syphilis, fungi (especially cryptococci), and carcinomatous meningitis¹⁵.

This study shows a significant increase in CSF WBCs count, CSF RBCs count, CSF protein levels, with a decrease in CSF glucose level for children with acute bacterial meningitis in comparison to the control group. Once in the CSF, the paucity of antibodies, complement components, and WBCs allow the bacterial infection to flourish. Bacterial cell wall components initiate a cascade of complement and cytokine-mediated events that result in increased permeability of the blood-brain barrier, cerebral edema, and presence of toxic

mediators in the CSF. Replicating bacteria, increasing numbers of inflammatory cells, cytokine-induced disruptions in membrane transport, and increased vascular and membrane permeability perpetuate the infectious process and account for the characteristic changes in CSF cell count, protein, and glucose¹⁶. Baker and Lenane (1989) showed that, in patients with bacterial meningitis, CSF white blood cell (WBC) counts are at least 300-2000 cells/mm¹⁷.

The pathologic whole mark of bacterial meningitis is subarachnoid space exudates, microscopically examination of this exudates in the early stage must demonstrate a large numbers of neutrophils and bacteria. Within 2-3 days of infection evidence of inflammation in the wall of the small and medium size subarachnoid blood vessels appeared as infiltration of lymphocytes and neutrophils in the wall of the meningeal veins which become distended with local necroses in the wall¹⁸.

The leakage of RBCs, WBCs, and proteins to the CSF may follow and it may contribute the increase numbers of CSF-RBCs, CSF-WBCs

and CSF-protein level, and these may represent the major and the first findings for the diagnosis of bacterial meningitis¹⁹. Dean et al (2003) showed an increase in CSF RBCs count for patients with bacterial meningitis²⁰.

As the typical CSF findings in acute bacterial meningitis consist of neutrophelic polycytosis, may be explained by the leakage of these cells from the focal necrotic lesions in the wall of subarrchenoid blood vessels, also the elevated CSF-protein concentration and the abnormal presence of RBCs in the CSF fluid may be attributed to the necrosis in the wall of subarrchenoid blood vessels, or these changes in CSF-RBCs, CSF-WBCs and CSF-protein level may be caused by the affection of the inflammation of the meninges on the normal barrier between the CSF and the blood circulation (i.e the blood-brain barrier BBB). Thomas et al (2003) stated that the CSF protein levels are above 150 mg/dL (>1.5 g/L) for those with bacterial meningitis⁶. However Several authors have demonstrated, that the CSF leukocytes count cannot reliably distinguish between bacterial and aseptic meningitis^{7,14}

Dean et al.²⁰ reported that CSF glucose concentration is expected to be below 40 mg/dL (<2.5 mmol/L), with a CSF:serum ratio of <0.25. This reduction in the CSF-glucose level may be the finding of the consumption of different types of cells accumulated in the CSF of patients with bacterial meningitis that not present in normal CSF.

This study demonstrates a significant increase in CSF AChE activity for children with bacterial meningitis, this finding is consisted with previous studies^{21,22}.

The destructions of several CSF-RBCs cause the release of

intraerythrocytic AChE to the CSF, or the leakage of plasma AChE across the abnormal BBB in case of meningitis can explain the increase of CSF-AChE activity in case of bacterial meningitis.

Despite the significant increase of CSF-AChE activity in meningitis group versus the control group, there is a reduction in the AChE specific activity in the meningitis group versus the control group. This finding show that the increase in the concentration of CSF-Protein is much higher than the increase in the activity of CSF-AChE among patients with bacterial meningitis, so still measurements of CSF-protein level are more important than the measurement of CSF-AChE and the measuring of CSF-AChE can not represent the measuring of CSF-protein, but can augment the other measures for the diagnosis.

In conclusions measurement of CSF- AchE activity in suspected cases of acute bacterial meningitis in children can help in the diagnosis and give an additional information to confirm the diagnosis specially for those with equivocal decision.

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