THE CHANGES IN SERUM ELECTROLYTES AND ENZYMES (AST, LDH AND CK-BB) IN PATIENTS WITH CNS INFECTIONS UNDER FIVE YEARS OLD.

A A Kadim¹, M J Ewadh², R M Hasani³.
¹Medical Laboratory Technician, Laboratory department, Neurosurgery Hospital, Baghdad, Iraq.
²Prof. Department of Biochemistry, College of Medicine, University of Babylon, Iraq.
³Asisst. Prof. Specialist, pediatric department, Babylon Maternity and children Hospital, Iraq.
¹ahmdali271990@gmail.com, ²Mewadh@yahoo.com, ³rebeemohsin@gmail.com

ABSTRACT

This study is designed to evaluate the level of serum electrolytes and the enzymes (AST, LDH and CK-BB) during October 2020 – January 2021 in Babylon state, Iraq. The aim is to observe the change in the concentration of those parameters in children under five years old with central nervous system (CNS) infections. 70 samples of blood have been collected from patients with CNS infection and 50 from normal individual as control group, 25 male (18.6 ± 1.2) and 25 female (18.3 ± 1.1). Hyponatremia was found in more than 70% of cases which most likely due to SIADH and rarely CSW, hypekalemia in 8.4% (n=6) and hypocalcemia in 56.3%. The concentration of Ck-BB isoenzyme was significantly (P≤ 0.01) higher in patient when compared with control group as well as the level of both AST and LDH in patient were significant high at (P≤ 0.01).

I. INTRODUCTION

In the past decade much research has focused on the changes in the level of electrolytes and enzymes in cerebral spinal fluid (CSF) to study the changing of these parameters and correlate them with the severity of the disorder as well as to find a significant change that may lead to rapid diagnosis of CNS infections. However, these disturbances may also be found in serum and can leads to serious complications and even death if not treated probably. The purpose of this study was to observe the changes in serum electrolytes and the enzymes: AST, LDH and CK-BB in children with CNS infections under five years old. Meningitis and encephalitis are the most frequent disorders of CNS infections. Meningitis is an inflammation of the brain meninges including the cerebrospinal fluid (CSF). It is categorized into: bacterial (pyogenic) meningitis(BM) and aseptic meningitis(AM) [1]. It caused by bacteria, parasites, fungi and the most commonly viruses. The mortality rate of meningitis is lower than 2% in children and 20-30% in newborn babies [2]. In BM, Hemophilus influenzae, Neisseria meningitides, and Streptococcus pneumoniae accounted for more than 80% of cases, S. pneumoniae the most common cause of meningitis [3]. AM or sometimes called viral meningitis (VM) is the inflammation of brain meninges with viruses, mostly Herpes viruses, or when patients presented with meningeal signs and symptoms and the laboratory results showed no bacterial growth in CSF [4]. There are four main ways of patient to have meningitis: 1st by direct inoculation of organism into the brain tissues or CSF, we see this often in head trauma and the infection mostly due to the bacterial [5], 2nd when the organism is present in the blood causing systemic or whole body infection, the organism can cross the barriers that exists between the blood and the brain tissues through variety of methods [6]. 3rd Microorganisms particularly viruses that directly infect nerve tissue and spread to the brain.[6] 4th Infection of sinuses surrounding the eyes can spread to the brain directly[7]. Encephalitis is the inflammation of brain parenchyma and sometimes associated with meningitis. It is a rare syndrome, up to 85% of global cases are of unknown etiology [8] but some viruses may contribute to cause it as; measles, mumps, polio, rabies, rubella and varicella (chicken box) and adenovirus [9]. In addition, there is autoimmune encephalitis(AIE) in which there is abnormal self-antibodies attach the cerebral tissues such as; Anti-NMDA receptor encephalitis[10]. Encephalitis caused by the HSV is the leading cause of more severe causes in all ages including neonates [11]. The viruses cause inflammation of the brain tissues, the brain tissues swell (cerebral edema) which may destroy the nerve cells causing
bleeding in the brain or intracranial hemorrhage and thus brain damage[12]. The level of Glucose in CSF during BM is very low, due to bacterial consumption, and protein is high. Cells are predominantly neutrophils and some lymphocytes maybe present. In VM the glucose level remains normal with high protein concentration[13]. Children with CNS infections usually develop dilutional hyponatremia due to inappropriate secretion of ADH (SIADH), extracellular volume depletion or rarely CSW syndrome [14]. The concentration of sodium below 134 mmol/L and in severe cases could be less than 125 mmol/L which associated with unconsciousness and convulsion[15]. The concentration of serum potassium K is normal in general but hypokalemia may also found in some patients with CNS infection most likely due to vomiting and reduce food intake[16]. Total and ionized hypocalcemia also associated with meningitis and/or encephalitis but total hypocalcemia is more frequent[17]. Creatine kinase brain-brain (CK-BB) isoenzyme is abundant in cerebral tissues and significantly increase in CSF during CNS disorders which indicate the extent of brain injury[18,19], however, it may leak out to the plasma due to increase the permeability and deterioration of the tight junction of blood brain barrier (BBB) resulting in an elevation plasma concentration this also explain the high level of serum AST and LDH in CNS infection[20,21].

II. METHODS AND MATERIALS

This study includes 70 samples from patient with CNS infections under five years old and 50 normal children during October 2020 – January 2021 from Babylon Maternity and children Hospital, Iraq. About 5ml of venous blood has been collected the blood has been left to clot in a gel tube then separated from cellular components by the centrifuge at 3000 (rpm) for 10 minutes, labeled the tube and stored at (- 20°C) for late biochemical measurements.

1.1 Determination of Serum LDH and AST.

Full automated Cobas C311 was used to determine the level of AST and LDH, which required specific reagents provided by Roche company. LDH catalyzes the conversion of L-lactate to pyruvate; NAD is reduced to NADH in the process.

\[
\text{L-Lactate} + \text{NAD}^+ \rightarrow \text{Pyruvate} + \text{NADH} + \text{H}^+
\]

AST catalyzes the transfer of an amino group between L-aspartate and a-ketoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH in the presence of malate dehydrogenase (MDH) to form NAD+. The rate of the NADH oxidation is directly proportional to the catalytic AST activity.

\[
L - \text{Aspartate} + \alpha - \text{keto glutarate} \leftrightarrow \text{oxaloacetate} + L - \text{malate}
\]

\[
\text{Oxaloacetate} + \text{NAD} + \text{H}^* \leftrightarrow L - \text{malate} + \text{NAD}^* + \text{H}^+
\]

Procedure

a) The reagents are pipetted by a fine needle from the reagent container into a transparent cuvette.

b) The serum drawn and mixed with the reagent by specific mixer to produce a homogenous concentration.

c) Incubate the solution at 37°C.

d) Enzyme activity will be measured photometrically after 10 minutes of incubation.

Roche/Hitachi Cobas C311 systems automatically calculate the analyte activity of each sample.

1.2 Determination of Serum Electrolytes

Electrolytes are measured customarily in Electrode Technology (Ion Selective Electrode “ISE”). In this technology, electrodes that contain membranes or glass calculate results by drawing the ions from the patient sample and comparing it to the ions inside the electrodes.

Procedure

a) Samples, calibrator and controls were prepared in small test tube and open the door led of the device.
b) The device will take few seconds to be ready.

c) The calibrator loaded first in order to calibrate the device.

d) After 120 seconds, each sample was loaded to the device. The results appeared on the screen for each sample after 1 minute.

1.3 Determination of CK-BB

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for CK-BB has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CK-BB present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for CK-BB is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CK-BB bound in the initial step. The color development is stopped and the intensity of the color is measured. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm.

III. STATISTICS

All statistical analyses were conducted using SPSS (version 26, IBM Corp., Armonk, NY, USA). Continuous data were determined to have a normal distribution if the Shapiro–Wilk test had a significance value of >0.05. Continuous variables were summarized using means and standard deviations or medians and interquartile ranges, as appropriate. Normal continuous data were evaluated using ANOVA. Correlations were measured via Spearman’s rank testing. Variables with a two-tailed p < 0.05 were considered to be significant.

IV. RESULTS

4.1 Serum electrolytes

The results of this study showed a significant decrease (P≤0.01) in the concentration of Na in patients (Mean=132.1 ± 1.3) when compared with control group (142.3 ± 0.3) (table-1). According to the reference range, the concentration of Na in 76.1% (n=54) of cases were below the normal level (hyponatremia) and 23.9% (n=17) were normal (table-2). The concentration of Na in encephalitis and meningitis showed no significant association (P=0.455). The results also showed a positive significant correlation with K and AST at (P≤ 0.01) (figure-1).

Table 1. level of Na⁺² in control and patient

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na⁺²(mmol/L) Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>142.3 ± 0.3</td>
<td>≤0.01</td>
</tr>
<tr>
<td>Patients</td>
<td>132.1 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Meningitis</td>
<td>130.9 ± 11.2</td>
<td>0.454</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>135.2 ± 6.5</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. The level of serum sodium in patients with CNS infections.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (135-145 mmo/l)</td>
<td>16</td>
<td>22.8%</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>53</td>
<td>75.7%</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>82.5%</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>17.5%</td>
</tr>
<tr>
<td>Mild (130-135 mmo/l)</td>
<td></td>
<td>50.7%</td>
</tr>
<tr>
<td>Moderate (129-125 mmol/L)</td>
<td></td>
<td>14.1%</td>
</tr>
<tr>
<td>Severe (less than 125mmol/l)</td>
<td></td>
<td>11.3%</td>
</tr>
<tr>
<td>Hypernatremia (more than 145)</td>
<td>1</td>
<td>1.5%</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>100%</td>
</tr>
</tbody>
</table>

Figure-1: The correlation between Na$^{+2}$ and K$^{+}$

The results of serum potassium showed non-significant differences between the mean of patients and control. Potassium level was normal in 77.1% (n=54) and slightly low (hypokalemia) in 17.1% (n=12) and 8.4% (n=6) was high (hyperkalemia). There is also a significant decrease in the concentration of serum Ca$^{+2}$ (0.9 ± 0.2) in comparison with control group (P≤ 0.01) without a significant association within age groups. There is no significant difference between ages and also the etiology of CNS infections (table-3).

Table-3: The level of K$^{+}$ and Ca$^{+2}$.

<table>
<thead>
<tr>
<th>Groups</th>
<th>K$^{+}$ Mean ±SD</th>
<th>Ca$^{+2}$ Mean ±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>4.0 ± 0.3</td>
<td>1.1 ± 0.02</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>patients</td>
<td>4.0 ± 0.7</td>
<td>0.9 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Meningitis</td>
<td>4.1 ± 0.7</td>
<td>0.9 ± 0.2</td>
<td>0.372</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>4.0 ± 0.5</td>
<td>1.02 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>
4.2 Serum enzymes.

The results of the present study revealed a significant increase in the concentration of AST in (29.6%) when compared with control group (P≤0.01) and 70.4% (n=50) was normal. The concentration of AST in encephalitis showed a significant increase (P≤0.01) in comparison with meningitis as well as a significant increase in BM than in VM. The study also showed a significant increase (P≤0.01) in the concentration of LDH (45.1% n=32) when compared with control group, Table-4. A significant increase in patients with encephalitis. There was also a significant variation between aseptic and bacterial meningitis (BM), the latter showed a significant increase (mean=453.1 ± 79.2), high serum LDH is strongly associated with the mortality of meningitis and/or encephalitis. The results also showed a positive significant correlation (r²= 0.489) between LDH and AST (P≤ 0.01) Figure-2. In this study it was observed that a significant increase in the concentration of serum CK-BB when compared with control group (mean= 36.3 ± 1.6) (P≤ 0.01). Neither gender nor the etiology of CNS infections showed significant differences. The study observed a negative significant correlation between CK-BB and Na+² (r= -0.190) as shown in Figure-3.

Figure 2. the correlation between AST and LDH.

Table-4: the level of serum AST, LDH and CK-BB.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>P value</th>
<th>Mean ± SD</th>
<th>P value</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST</td>
<td></td>
<td>LDH</td>
<td></td>
<td>CK-BB</td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>33.7 ± 1.3</td>
<td>≤0.01</td>
<td>373 ± 30.2</td>
<td>≤0.01</td>
<td>36.3 ± 1.6</td>
<td>≤0.01</td>
</tr>
<tr>
<td>Control</td>
<td>45.3 ± 4.1</td>
<td></td>
<td>277.6 ± 11.4</td>
<td>≤0.01</td>
<td>26.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Meningitis</td>
<td>45.1 ± 4</td>
<td>≤0.01</td>
<td>366.9 ± 31.9</td>
<td>≤0.01</td>
<td>36.3 ± 1.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>47.4 ± 18.2</td>
<td></td>
<td>482.3 ± 86.1</td>
<td></td>
<td>35.2 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>B.M</td>
<td>38.4 ± 6.3</td>
<td>≤0.01</td>
<td>453.1 ± 79.2</td>
<td>≤0.01</td>
<td>38.4 ± 2.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>V.M</td>
<td>35.2 ± 4</td>
<td></td>
<td>358.9 ± 32.6</td>
<td></td>
<td>33.6 ± 2.2</td>
<td></td>
</tr>
</tbody>
</table>
Figure-3: the correlation between CK-BB and Na.

V. DISCUSSIONS

a. Serum electrolytes

This study showed that patients with neurological disorder usually associated with hyponatremia in 75.7% of cases, the majority of whom classified as mild. As expected, severe and moderate hyponatremia were related with the occurrence of certain symptoms (convulsion and conscious symptoms). Similar results have also been reported in an article published by the Department of Neurology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India. [15]. There are two main conditions responsible for most cases of hyponatremia in patients with neurological disease: Cerebral Salt Wasting syndrome “CSW” and Syndrome of inappropriate secretion of anti-diuretic hormone (SIADH)[22,23]. SIADH is more frequently seen in meningitis and encephalitis[24]. The concentration of potassium in patients with neurological disorder is variable, some high and others below the normal range [25]. However, Tow patients with acute encephalitis had increased plasma potassium and remarkable reduction in serum sodium died within 72 hr of admission. Zheng and Jiang, L 2019 find similar results[26]. Hypokalemia in meningitis may result from abnormal high level of ADH which increases renal distal tubular secretion[27,28]. Hypocalcemia is a decrease in the concentration of both total and ionized serum calcium which commonly seen in children with meningococcal disease[29]. Gauthier et al find out that 14% in 45 critically ill children had ionized hypocalcemia[17], Cardenas-Rivero et also reported an ionized hypocalcemia in 18% [30] while Zaloga demonstrate that 70-90% had a total hypocalcemia and 15-50% had an ionized hypocalcemia in children who have meningococcal disease[31].

b. Serum enzymes

Serum AST enzyme was significantly high when compared with control group, and a significant increase in encephalitis, Ning Cui, find a significant increase of AST in encephalitis which used, in part, to monitor the severity of the disease [32]. The concentration was significantly higher in B.M than V.M [33]. High AST concentration is usually associated with the severity of the disease and death, this is in agreement with the result of other study that reported an association between high AST and death in children with CNS infections[34]. High serum LDH is strongly associated with the mortality of meningitis and/or encephalitis this is matched with Nand N, Sharma M, who reported a significant increase in serum LDH in 3 cases who died eventually[35]. High serum LDH in children with CNS infections may originated from cellular damage due to viral or bacterial infection[36] or sometimes liver involvement may contribute to release more LDH, which is not related to CSF level[37]. Some studies reported that the raised in serum LDH activity cannot be due disturbed BBB because LDH activity is normally low in CSF when compared to serum [38]. High level of serum CK-BB indicate the extent of brain injury[18]. Theoretically, any injury in organs that contain CK isoenzyme should release soluble enzymes into the circulation[39]. However, the source of serum CK-BB is not clear but it could be excreted from CSF during acute phase of inflammation[40]. Tadele, F, suggested that CK may leak out from the damaged cerebral cells during CNS infection into the circulation[41]. On the other hand, Nussinovitch, M. et, reported that there is no clear evidence confirming that the source of serum CK-BB is the brain tissues[42].
Central nervous system infections are usually associated with serum electrolytes disturbance predominantly hyponatremia, most of whom had mild hyponatremia which is more sever in encephalitis. Hyperkalemia in CNS infections may result from hemolyzed or old blood sample resulting in a small shift of K out of cells and cause a significant raise in serum concentrations or may results from accompanied high ADH activity. Patients with encephalitis usually at high risk of death and showed severe electrolytes and enzyme disturbance. Patients with bacterial meningitis showed higher incidence of serum electrolytes and enzymes abnormalities. Most patients showed normal concentrations of serum enzymes but those with high level usually associated with the severity of the disease except for CK-BB.

Acknowledgments

I am thankful to Prof. Dr. Mufeed J. Ewadh and Asisst. Prof. Dr. Rabee M. Hassanii for their priceless supports.

REFERENCES