STUDYING THE ENZYMES LEVELS IN CEREBROSPINAL FLUID FOR CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT

The research includes studying of enzymes levels in the cerebrospinal fluid (CSF) for children with acute lymphoblastic leukemia (ALL) by determination of (9) parameter which include biochemical parameters: Acetylcholinesterase (AChE), Pseudocholinesterase (PChE), Monoamine oxidase A (MAOA), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Creatinine kinase (CK), Lactate dehydrogenase (LDH) and Superoxide dismutase (SOD). The study was done on (140) sample had acute lymphoblastic leukemia (95) were male and (45) female, beside of (73) were healthy children as control group which include (39) male and (34) female the age of children with acute lymphoblastic leukemia ranged from 1-15 year for both sex. The results showed there was a significant decrease in the activity of AChE, PChE and a significant increase in MAOA, AST, ALT, ALP, CK, LDH and SOD in CSF for children with acute lymphoblastic leukemia patients compared with control group for both sexes and these were a very good biochemical indicators for the patient body biochemical functions. The blood brain barrier loss its function in maintaining brain homeostasis because of the oxidative stress and the age group (11-15)y of male had higher oxidative stress.

KEYWORDS: Acute lymphoblastic leukemia, Cerebrospinal fluid, Enzymes.

INTRODUCTION

Leukemia is a group of disorders characterized by the accumulation of malignant white cells in the bone marrow and blood. These abnormal cells cause symptoms because of: bone marrow failure (e.g anemia, neutropenia, thrombocytopenia) and infiltration of organs (e.g.
liver, spleen, lymph nodes, meninges, brain, skin, test).[1] Clinically and pathologically, leukemia is subdivided into a variety of large groups. The first division is between its acute and chronic forms.[2] Additionally, the diseases are subdivided according to which kind of blood cell is affected. This divides leukemia’s into lymphoblastic or lymphocytic leukemia’s and myeloid or myelogenous leukemia’s. Combining these two classifications provides a total of four main categories: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML).[3]

Cerebrospinal fluid (CSF) is considered a part of the trans cellular fluids. It is contained in the ventricles and the subarachonid space and bathes the brain and spinal cord. The CSF is contained within the meninges and acts as a cushion to protect the brain from injury with position or movement. CSF is a clear, colorless, body fluid found in the brain and spine produced the choroid plexuses of the ventricles of the brain and serves several purposes as buoyancy, protection, chemical stability, prevention of brain ischemia and clearing waste. The CSF also serves a vital function in cerebral auto regulation of cerebral blood flow.[4]

Cholinesterase is a family of enzymes that catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation. It involves two types: (Acetylcholinesterase (AChE) and Pseudocholinesterase (PChE) the difference between the two types has to do with their respective preferences for substrates[5]. Many studies have found that the expression level of AChE increases during apoptosis in various cell types.[6]

The monoamine oxidases (MAOs) are mitochondrial bound isoenzymes which catalyze the oxidative deamination of monoamine neurotransmitters, including 5-hydroxytryptamine, histamine and catecholamines (Dopamine, noradrenaline and adrenaline). MAO is classified into two types (A and B), according to their sensitivity towards specificity substrates and inhibitors.[7]

Different aminotransferases show different tissue distribution: aspartate aminotransferase activity is high across most tissues, whereas alanine aminotransferase activity is highest in the liver.[8] The alkaline phosphatase affects the inflammatory responses and may play a direct role in preventing organ damage, for example it affect on the inflammatory responses in patients with chronic kidney disease and is directly associated with erythropoiesis stimulating agent resistant anemia.[9] Importantly, previous studies have demonstrated that creatinine kinase may play an important role in immune response, including adaptive immune response.
and innate immune response. A previous study has demonstrated that CK-BB is an important regulator of T cell development and activation via TCR signaling.\cite{10} The LDH is involved in tumor initiation and metabolism. Cancer cells rely on anaerobic respiration for the conversion of glucose to lactate even under oxygen-sufficient conditions.\cite{11}

SOD participate in compartmentalized redox signaling to regulate many vascular function. Dysregulation of these signaling pathways leads to endothelial dysfunction, altered vascular tone, vascular inflammation, vascular remodeling, enhanced vascular permeability and increased platelet aggregation, which contribute to impaired angiogenesis as well as various vascular diseases such as atherosclerosis and hypertension.\cite{12}

**MATERIAL AND METHOD**

The study was done on (140) sample had acute lymphoblastic leukemia (95) were male and(45) female and (73) were healthy children as control group(39) male and (34) female the age of children with acute lymphoblastic leukemia ranged from 1- 15 years for both sex. The samples of CSF were collected by specialized doctors and the method was lumbar puncture (LP). Different questions asked for patients and control groups that include medical history, duration of leukemia, acute illness, weight, height and drugs usage. The biochemical test included each of AChE, PChE, MAOA, SOD were manually measured while AST, ALT, CK, ALP and LDH were measured using the standard kits from Biolabo.

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>The method</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE</td>
<td>S-Acetylthiocholine Iodide method\cite{13}</td>
</tr>
<tr>
<td>PChE</td>
<td>Buterylthiocholine Iodide method\cite{13}</td>
</tr>
<tr>
<td>MAO</td>
<td>Oxidize benzylamine method\cite{14}</td>
</tr>
<tr>
<td>AST and ALT</td>
<td>Reitman and Rankel method\cite{15}</td>
</tr>
<tr>
<td>ALP</td>
<td>Kind and King method\cite{16}</td>
</tr>
<tr>
<td>CK</td>
<td>Oliver method\cite{17}</td>
</tr>
<tr>
<td>LDH</td>
<td>NADH Colorimetric method\cite{18}</td>
</tr>
<tr>
<td>SOD</td>
<td>Modified photochemical nitrobluetetrazolum (NBT) method\cite{19}</td>
</tr>
</tbody>
</table>

**STATICAL ANALYSIS**

In this study the results include Mean ± SD and significant differences (p value) between groups that examined by a available statistical (SPSS 17.0), significant differences was estimated as the p value was equal or less than 0.05.\cite{20}
RESULTS AND DISCUSSIONS

1- Acetylcholinesterase (AChE)

ACHE activity was found to be significant decreased (p≤0.05) in CSF of patients with acute lymphoblastic leukemia compared with control group as shown in tables (2) and (3) in both sexes for all age groups and it was higher in male than female the lowest activity was found in the (11-15) years age group for male, the result was in agreement with other reports which found a lower activity of the enzyme in serum of leukemia patients and in serum for patients with lung cancer. AChE is a novel regulator in cell proliferation and cell death, allowing AChE as a potential marker for cancer diagnosis and prognosis. AChE gene expression is always decreased in tumor tissues, and AChE has been reported to suppress cell proliferation via catalytic hydrolysis of acetylcholine, So acts as a tumor growth suppressor. The decrease in AChE activity and the consequent increased level of acetylcholine could cause cholinergic overstimulation and enhance the cell proliferation in cancer. It has also been demonstrated that AChE can hydrolyze lipid peroxides and that may support the possibility of the reduction in enzyme activity augments oxidative stress and cellular damage. The effect of estrogen on the brain and its stimulation to pull of choline and further build the acetylcholine, may be the reason for the higher activity of enzyme in male than female.

2- Pseudocholinesterase (PChE)

PChE activity was found to be significant decreased (p≤0.05) in CSF of patients with acute lymphoblastic leukemia compared with control group as shown in tables (2) and (3) in both sexes for all age groups and it was higher in male than female the lowest activity was found in the (11-15) years age group for male, the result was in agreement with other reports. which showed a decrease in enzyme activity in serum of liver disorders patients.

One of the possible mechanisms responsible for PChE activity decrease in cancer patients could be secondary anorexia accompanying malignancy. In cancer, with or without liver impairment, stress starvation stimulates an inflammatory cascade which blocks the synthesis of PChE, albumin and other visceral proteins in favor of acute-phase protein synthesis. In these conditions, serum PChE results an accurate functional and prognostic indicator, useful for monitoring the clinical and therapeutic intervention according to the patient's survival expectancy.
Table (2): The activity of AChE, PChE and MAOA enzymes in CSF for female with ALL.

<table>
<thead>
<tr>
<th>Activity of enzymes</th>
<th>Age groups (Mean + SD)</th>
<th>Age group (1-5) y</th>
<th>Age group (6-10) y</th>
<th>Age group (11-15) y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Patients</td>
<td>Control</td>
<td>Patients</td>
</tr>
</tbody>
</table>

| AChE nmol/ml/min | 9.54 ± 1.24 | 5.23 ± 0.47* | 9.4 ± 1.05 | 4.92 ± 1.05* | 9.22 ±1.15 | 4.1 2 ±1.1** |
| PChE nmol/ml/min | 69.32 ±7.8 | 40.25 ±8.92* | 68.32 ±7. 2 | 43.67 ±4.21* | 67.51 ±8.21 | 38.61 ±5.5* |
| MAOA U/L | 44.05 ±10.3 | 131.7 ±16.9** | 41.3 ±9.6 | 98 ± 12.34 ** | 48.12 ±8.6 | 164.6 ±18.8** |

*Significant difference at p<0.05.
** Significant difference at p<0.001.

Table (3): The activity of AChE, PChE and MAOA Enzymes in CSF for male with ALL.

<table>
<thead>
<tr>
<th>Activity of enzymes</th>
<th>Age groups (Mean + SD)</th>
<th>Age group (1-5) y</th>
<th>Age group (6-10) y</th>
<th>Age group (11-15) y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Patients</td>
<td>Control</td>
<td>Patients</td>
</tr>
</tbody>
</table>

| AChE nmol/ml/min | 9.06±1.04 | 5.33±1.1* | 8.9±1.39 | 4.65±0.96* | 8.87±1.1 | 4.5±0.78* |
| PChE nmol/ml/min | 69.33±2.9 | 46.66±6.8* | 68.26±9.6 | 42.25±6.21* | 65.67±11.7 | 41.67±5.52* |
| MAOA U/L | 46.21±11.8 | 141.34±20.00** | 42.25±7.8 | 128±10.5** | 49.12±11.6 | 142.6±7.8** |

*Significant difference at p<0.05.
** Significant difference at p<0.001.

3- Monoamine oxidase A (MAOA)

MAOA activity was found to be significant increased (p<0.05) in CSF of patients with acute lymphoblastic leukemia compared with control group as shown in tables (2) and (3) in both sexes for all age groups, this result was in agreement with other reports\textsuperscript{[27,28]} who found evaluated levels of MAO in prostate cancer patients.

MAOA is a key enzyme involved in the degradation of the biogenic and dietary monoamine neurotransmitters such as 5-hydroxytryptamine (5-HT, or serotonin) and norepinephrine by oxidative deamination. Amine metabolism is linked to essential cellular processes such as cell growth and differentiation, and the catalytic byproducts of MAOA, such as hydrogen peroxide, are known to cause oxidative damage with implications for cancer, aging and neurodegenerative processes. hydrogen peroxide a major source of ROS which can predispose cancer cells to DNA damage and cause tumor initiation and progression.\textsuperscript{[29]}

4 Aminotransferase enzymes (ALT, AST)

Our study found that there was a significant increase (p<0.05) in CSF ALT and AST activity
of patients with acute lymphoblastic leukemia compared with control group as shown in tables (4) and (5) and this result was in agreement with previous studies which showed a significant increase in both enzymes in serum of patients with leukemia.\cite{30,31} Viral hepatitis, Liver cirrhosis\cite{24} the evaluation of these enzymes in leukemia patients may be due to hepatic infiltration. In infiltrative disorder as a result of a defect in the membranes of mitochondria and cytoplasm which is proportional with increased of AST.\cite{30} Increase level of ALT and AST enzyme are popular at initial appeared of leukemia and that lead to hepatic injury from leukemic stream\cite{32} that elevated of leukemic cell lead to increase of transaminase enzyme concentration\cite{33} because the toxicity of chemotherapy treatment.\cite{30}

In experimental models, the capacity of the enzyme AST to remove glutamate from the brain by means of blood glutamate degradation has been shown to be an efficient and novel neuro protective strategy against neuro disorder damage.\cite{34} It has been well established that abnormally high concentrations of glutamate in the interstitial fluid and CSF of the brain are associated with several neurodegenerative conditions.\cite{35} GOT1 expression correlates with the growth of several tumors because cancer cells can utilize the amino acid glutamine to fuel anabolic processes and therefore, GOT1 represents a new therapeutic target in cancer.\cite{36}

5 Alkaline phosphatase (ALP)

The results in tables (4) and (5) shows a significant increase (P≤0.05) in ALP activity in CSF of leukemia patients for both sexes and all age group compared with the control group and the enzyme activity was in male higher than female and its proportional with age and this was in agreement with Turan et al.\cite{37} Several studies reported elevation of ALP activity in early stages of patients serum of ovarian cancer, breast cancer, prostate cancer, brain cancer, esophageal cancer, colorectal cancer and lung cancer when compared with normal controls\cite{38} and the elevation in ALP activity occurs because of the accelerated denovo synthesis of the enzyme\cite{40} or because the increase of tumor necrosis factor-α and its direct effect on the expression of alkaline phosphatase in vascular smooth muscle cells as well as how ALP affects the inflammatory responses and may play a direct role in preventing organ damage.\cite{41}
Table (4): The activity of AST, ALT, ALP, CK, LDH and SOD enzymes in CSF for female with ALL.

<table>
<thead>
<tr>
<th>Activity of enzymes</th>
<th>Control (Age group (1-5) y)</th>
<th>Patients (Age group (1-5) y)</th>
<th>Control (Age group (6-10) y)</th>
<th>Patients (Age group (6-10) y)</th>
<th>Control (Age group (11-15) y)</th>
<th>Patients (Age group (11-15) y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST U/L</td>
<td>7.75 ±0.95</td>
<td>14.0 ±0.82*</td>
<td>8.63 ±1.25</td>
<td>15.1 ±1.7*</td>
<td>8.5 ±1.4</td>
<td>14.8 ±1.5*</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>4.75 ±1.5</td>
<td>10.75 ±2.10*</td>
<td>5.4 ±1.48</td>
<td>10.6 ±3.37*</td>
<td>5.4 ±1.8</td>
<td>11.1 ±3.1*</td>
</tr>
<tr>
<td>ALP U/L</td>
<td>8.1 ±0.85</td>
<td>11.5 ±0.35*</td>
<td>9.07 ±1.72</td>
<td>13.2 ±1.16*</td>
<td>9.2 ±1.86</td>
<td>14.4 ±2.76*</td>
</tr>
<tr>
<td>CK U/L</td>
<td>2.94 ±0.61</td>
<td>4.9 ±0.92*</td>
<td>3.11 ±0.19</td>
<td>5.52 ±0.35*</td>
<td>3.04 ±0.52</td>
<td>6.2 ±0.84*</td>
</tr>
<tr>
<td>LDH U/L</td>
<td>55.6 ±5.3</td>
<td>88.2 ±5.2**</td>
<td>57.4 ±4.3</td>
<td>86.4 ±6.2**</td>
<td>59.4 ±5.8</td>
<td>92.4 ±4.8**</td>
</tr>
<tr>
<td>SOD U/L</td>
<td>0.25 ±0.06</td>
<td>0.602 ± 0.09*</td>
<td>0.32 ±0.04</td>
<td>0.63 ±0.081*</td>
<td>0.33 ±0.026</td>
<td>0.65 ± 0.083*</td>
</tr>
</tbody>
</table>

*Significant difference at p<0.05.
** Significant difference at p<0.001.

Table (5): The activity of AST, ALT, ALP, CK, LDH and SOD enzymes in CSF for male with ALL.

<table>
<thead>
<tr>
<th>Activity of enzymes</th>
<th>Control (Age group (1-5) y)</th>
<th>Patients (Age group (1-5) y)</th>
<th>Control (Age group (6-10) y)</th>
<th>Patients (Age group (6-10) y)</th>
<th>Control (Age group (11-15) y)</th>
<th>Patients (Age group (11-15) y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST U/L</td>
<td>8.6 ±1.24</td>
<td>14.5 ± 1.43*</td>
<td>8.25±1.5</td>
<td>14.2±0.96*</td>
<td>8.35±1.31</td>
<td>15.95±1.4*</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>4.72 ±0.83</td>
<td>8.25 ±2.69*</td>
<td>5.1 ±2.1</td>
<td>9.66±1.24*</td>
<td>5.5 ±1.7</td>
<td>12.0±3.3*</td>
</tr>
<tr>
<td>ALP U/L</td>
<td>9.0 ± 0.89</td>
<td>12.5±0.54*</td>
<td>9.15±1.2</td>
<td>13.62±2.3*</td>
<td>9.7 ±1.17</td>
<td>16.0±1.5*</td>
</tr>
<tr>
<td>CK U/L</td>
<td>3.1 ± 0.58</td>
<td>5.75±0.84*</td>
<td>3.2 ±1.48</td>
<td>6.45±0.57*</td>
<td>3.58±0.22</td>
<td>6.8±0.44*</td>
</tr>
<tr>
<td>LDH U/L</td>
<td>56.2 ±5.2</td>
<td>90.2±4.2**</td>
<td>58.4±4.3</td>
<td>96.4±5.2**</td>
<td>60.6±6.8</td>
<td>102±7.7**</td>
</tr>
<tr>
<td>SOD U/L</td>
<td>0.27±0.03</td>
<td>0.75±0.07*</td>
<td>0.3 ±0.05</td>
<td>0.77±0.15*</td>
<td>0.34±0.04</td>
<td>0.85±0.09*</td>
</tr>
</tbody>
</table>

*Significant difference at p<0.05.
** Significant difference at p<0.001.

6 Creatine kinase (CK)

CK activity was found to be significant increased (p≤0.05) in CSF of patients with acute lymphoblastic leukemia compared with the control group as shown in tables (4) and (5) in both sexes for all age groups, it was higher in male than female the higher activity was found in the (11-15) years age group for male this result was in agreement with other reports[42]
which showed a high activity in serum of chronic leukemia patients. The appearance of creatine kinase (CK) in blood has been generally considered to be an indirect marker of muscle damage, particularly for diagnosis of medical conditions such as myocardial infarction, muscular dystrophy and cerebral diseases.\textsuperscript{[43]} Metabolic muscle disturbance is thought to result in release of cellular components through a cascade of events, which begin with depletion of ATP and result in the leakage of extracellular calcium ions into intracellular space, due to both Na-K-ATPase and Ca\textsuperscript{2+}-ATPase pump dysfunction. Intracellular proteolytic enzyme activity can increase and promote muscle protein degradation and augmented cell permeability, which allows some cell contents to leak into the circulation\textsuperscript{[44]} we also noticed that the activity of CK is proportional with age this means that tissues become more damaged as age progresses\textsuperscript{[45]} the high activity of CK in male may be due to the genetic effect of k70 which enhance the release of CK.\textsuperscript{[46]}

7 Lactate dehydrogenase (LDH)
LDH activity was found to be significant increased (p\leq0.05) in CSF of patients with acute lymphoblastic leukemia than control as shown in tables (4) and (5) in both sexes for all age groups, it was higher in male than female the higher activity was found in the (11-15) years age group for male this result was in agreement with other reports which showed high activity of enzyme in serum of patients with lymphoma, prostate cancer, renal cell carcinoma (RCC) and melanoma\textsuperscript{[11]}, colorectal cancer.\textsuperscript{[47]} LDH is up regulated in human cancers, and is associated with aggressive tumor outcomes.\textsuperscript{[48]} One of the principal biochemical characteristics of malignant cells compared to normal cells is a metabolic switch from oxidative phosphorylation to increased glycolysis, even under hypoxic conditions and is termed the Warburg effect the lactate dehydrogenase A (LDHA) catalyzes the conversion of pyruvate to lactate and is considered to be a key checkpoint of anaerobic glycolysis.\textsuperscript{[11]} It is elevated in many types of cancers and has been linked to tumor growth, maintenance, and invasion.\textsuperscript{[48]} The origin of the increased LDH in the CSF of patients with a pathology of the central nervous system is not understood. Some authors have suggested a disturbance in the brain barrier which permits plasma LDH to reach the CSF, or LDH production by neoplastic tissue or white blood cells and exogenous bacterial sources. However, neither of these has yet been proven. The spinal fluid level varies independently of the plasma or serum activity.\textsuperscript{[49]}
8 Superoxide dismutase (SOD)

The results in tables (4) and (5) showed that there was a significant increase (p<0.05) in superoxide dismutase activity in CSF of acute lymphoblastic leukemia patients in both sexes and for all age group, our result was in agreement with previous studies which showed a significant increase in SOD activity In serum for patients with Chronic myeloid leukemia (CML)\textsuperscript{50} and acute myeloid leukemia (AML)\textsuperscript{51} and in CSF of children with neurocysticercosis\textsuperscript{52}. The most efficient enzymatic antioxidants is superoxide dismutase (SOD), SOD activity protects cells from free radicals induced injury by catalyzing the dismutation of O\textsuperscript{2-} to O and to the less-reactive species hydrogen peroxide (H\textsubscript{2}O\textsubscript{2})\textsuperscript{53}. Superoxide dismutase (EC 1.15.1.1) is an enzyme that alternately catalyzes the dismutation the superoxide (O\textsubscript{2-}) radical into either ordinary molecular oxygen (O\textsubscript{2}) or hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). Superoxide is produced as a by- product of oxygen metabolism and, if not regulated, causes many types of cell damage, hydrogen peroxide is also damaging, but Less than Superoxide radical and it is degraded by other enzymes such as catalase, Thus, SOD is an important antioxidant defense in nearly all living cells exposed to oxygen\textsuperscript{54}.

CONCLUSION

1- There was a high oxidative stress in CSF of patients with acute lymphoblastic leukemia.
2- The blood brain barrier loss its function in maintaining brain homoeostasis because of the oxidative stress.
3- The age group (11-15)y of male had higher oxidative stress.

RECOMMENDATIONS

1-The possibility of using the enzymes AChE, PChE and MAOA, as biochemical parameters to follow the severity of the disease.
2-Conducting studies in the CSF to estimate more biochemical parameters and study its relation with the disease.

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