Study of Some Biochemical Parameters in Iraqi Children with Acute Lymphoblastic Leukemia

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Abstract:
Leukemia or cancer of the blood is the most common childhood cancer. Acute lymphoblastic leukemia (ALL), is the most common form of leukemia that occurs in children. It is characterized by the presence of too many immature white blood cells in the child’s blood and bone marrow. Acute lymphoblastic leukemia can occur in adults too, treatment is different for children. Children with ALL develop symptoms related to infiltration of blasts in the bone marrow, lymphoid system, and extramedullary sites, such as the central nervous system (CNS). Common constitutional indications consist of fatigue (50%), pallor (25%), fever (60%), and weight loss (26%). Infiltration of blast cells in the marrow cavity and periosteum often lead to bone pain (23%) and disturbance of normal hematopoiesis. Thrombocytopenia with platelet counts less than 100,000 are seen in approximately 75% of patients. About 40% of patients with childhood ALL present with hemoglobin levels less than 7 g/dL. Although leukocyte counts greater than 50,000/mm³ occur in 20% of cases, neutropenia defined as an absolute neutrophil count less than 500 is common at presentation and is associated with an increased risk of infection. The aim of this study was to investigate the differentiations in some biochemical parameters (Hb, PCV, total serum proteins Aspartate aminotransferase(AST), Alanin amino transferase (ALT), and Malondialdehyde (MDA) in blood which can be conceded as a marker of ALL. Samples were collected from 50 patients (between 1-16 years old) diagnosed with ALL after one month treatment with induction therapy, compared with 30 control samples taken from healthy persons at the same age.

The ALT and MDA showed a significant increase \( p < 0.001 \) and \( p<0.01 \) respectively, in patients group compared to control group. There was a negative correlation between ALT [IU/l] with PCV % in Patients group \( (r = 0.22 \), \( p<0.05 \), while there was no significant correlation observed in the control group. The current study concluded that elevated levels of ALT and MDA levels at the diagnosis may be due to the side effect of induction therapy treatment an unfavorable result in ALL Iraqi child.

Keywords: Acute lymphoblastic leukemia (ALL), AST, MDA.

Introduction:
Acute lymphoblastic leukemia is a cancer of the blood and bone marrow (spongy tissue in the center of bone). In ALL, too many bone marrow stem cells develop into a type of white blood cell called lymphocytes. These abnormal lymphocytes are not able to fight infection very well. Also, as the number of these lymphocytes increases, there is less room for healthy white blood cells, red blood cells, and platelets. [1].

Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children. About 80% of children with leukemia have acute

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lymphoblastic leukemia [2], it is a malignant proliferation of lymphoid cells blocked at an early stage of differentiation. Although it may affect children of any age, there is a peak modal distribution between 3 and 6 years. The presenting features may be quite variable but in most case the diagnosis is promptly reached by morphological examination of a bone marrow aspirate[3]. The effects of ALL include uncontrolled and exaggerated growth and accumulation of cells called “lymphoblasts” or “leukemic blasts,” which fail to function as normal blood cells[4]. The presence of the leukemic blasts blocks the production of normal cells. As a result, when ALL is diagnosed, the number of healthy blood cells (red blood cells, white blood cells and platelets) is usually lower than normal[5].

ALL is a biologically heterogeneous disorder, so that morphologic, immunologic, cytogenetic, biochemical, and molecular genetic characterizations of leukemia lymphoblast are needed to establish the diagnosis or to exclude other possible causes of bone marrow failure and, finally, to classify ALL subtypes [6].

Children with acute lymphoblastic leukemia (ALL) often present with signs and symptoms that reflect bone marrow infiltration and/or extramedullary disease. When leukemic blasts replace the bone marrow, patients present with signs of bone marrow failure, including anemia, thrombocytopenia, and neutropenia[7]. It is widely accepted that solid tumor cells have an altered metabolic profile. First described by Otto Warburg in 1956[8], the cells of solid tumors have been shown to have elevated rates of glucose transport and glycolysis compared to their nonmalignant counterparts. This increase in glycolysis results in the production of copious amounts of lactate, even in the presence of oxygen as the result of reduced tricarboxylic acid (TCA) cycle function[9].

Anemia, abnormal leukocyte and differential counts, and thrombocytopenia are usually present at diagnosis, reflecting the degree to which bone marrow has been replaced with leukemic lymphoblasts. Anemia is common in patients with newly diagnosed childhood acute lymphoblastic leukemia (ALL). Approximately 40% of patients with childhood ALL present with hemoglobin levels less than 7 g/dl [10-13]. Several studies have demonstrated a correlation between degree of anemia and survival[14-15]. The hematocrit (Ht or HCT) or Packed cell volume (PCV) is the percentage of blood volume that is occupied by red blood cells. It is normally approximately 45% for men, 40% for women. The levels change with age, sex and general health [16].

Elevated transaminases are common at initial presentation of ALL and are likely due to hepatic injury from leukemic infiltrates. Conjugated hyperbilirubinemia at presentation may require treatment modification and dose reduction [17].

Defense mechanisms of the body play an important role in the form of anti-oxidants and therefore, minimize the damage, adapting itself to the stressful situations. Antioxidants are com-pounds that dispose, scavenge, and suppress the formation of ROS, or oppose their actions and play a major role in the prevention of various diseases including cancer and their clinical manifestations. Lipid peroxidation is evaluated in terms of malondialdehyde (MDA) levels [18].

The generation of reactive oxygen species (ROS) and other free radicals (R) during metabolism is a necessary
and normal process that ideally is compensated for by an elaborate endogenous antioxidant system. However, due to many environmental, lifestyle, and pathological situations, excess radicals can accumulate, resulting in oxidative stress. Oxidative stress has been related to cardiovascular disease, cancer, and other chronic diseases that account for a major portion of deaths today. Antioxidants are compounds that hinder the oxidative processes and thereby delay or prevent oxidative stress. This article examines the process of oxidative stress and the pathways by which it relates to many diseases[19]. Vitamin C is one of the important and essential vitamin for human health. It is needed for many physiological functions in human biology, vitamin C is a six-carbon lactone that is synthesized from glucose in the liver of most mammalian species, but not by humans[20,21]. In humans, vitamin C acts as an electron donor for eight different enzymes [22]. All known physiological and biochemical actions of vitamin C are due to its action as an electron donor[23,24]. Many health benefits have been attributed to ascorbic acid namely antioxidant, anti-atherogenic and anti-carcinogenic activity[25]. Malondialdehyde (MDA), one of the well-known secondary products of lipid peroxidation after exposure to reactive oxygen species and free radicals, may be used as an indicator of cell membrane injury [26].

Acute renal failure (ARF) is a well-recognized complication of acute leukemia. In acute leukemia, renal complications occur due to several factors including preexisting disorders, nephrotoxic drugs, sepsicaemias, leukaemic infiltration of the kidneys and therapy-related side effects such as tumourlysis syndrome. ARF may present at the time of diagnosis. However, primary manifestation of leukemia rarely occurs in the kidney [27].

The aim of this study was to investigate the differentiations in some biochemical parameters (Hb, PCV, total serum proteins Aspartate amino transferase (AST), Alanin amino transferase (ALT), and Malondialdehyde (MDA) in blood which can be conceded as a marker of ALL.

Materials and Methods:

Blood samples were collected from 50 patients diagnosed from Iraqi child with (ALL) as they were submitted to the Protection of Children Hospital Medical City in Baghdad, Iraq.

The diagnosis for ALL based on the following findings: age, leukocyte count, involvement of tissues other than bone marrow. Age and sex matched 30 healthy persons who are devoid of conditions like diabetes mellitus, epilepsy, psychiatric disorders or history of any drug intake are selected as control. Five ml of venous blood was drawn from (50) patients of ALL ranging between (1-15) years old, after 30 days induction therapy treatment and (30) normal control. The blood was allowed to clot for at least 20 min. at room temperature, centrifuged for (15) min. at (3500xg). Serum was removed and was stored at -18 °C until the time of measure the biochemical parameters. Serum ALL, ASL activity, glucose, creatinine, urea and protein concentrations were measured by using BIOMAGHRIB Kit. Plasma malondialdehyde (MDA) was determined according to the modified method of Satoh (1978) [28].

Statistical analyses of this study were performed using SPSS version 15.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL,.
USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability $P<0.05 = \text{significant}, P > 0.05 = \text{non-significant}$.

Results and Discussion:

There is no significant different in age glucose, urea ,AST, creatinine, and vitamin C between leukemia patients group and normal group. There were a significant decrease in Hb , PCV , total protein ,while MDA , and ALT were found to be significantly increase with $p < 0.001$ and $p<0.01$ respectively , in patients group when compared to control group as shown in table 1.

Table 1: The mean and standard deviation of Hb , PCV , protein ,glucose ,urea ,creatinine ,AST, ALT , MDA & vitamin C in leukemia patients group and control group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients group mean ± SD N=50</th>
<th>Control group mean ± SD N=30</th>
<th>$P$ Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [year]</td>
<td>15.47±3.96</td>
<td>15.3±4.97</td>
<td>N.S</td>
</tr>
<tr>
<td>Hb [g/dl]</td>
<td>9.11±1.42</td>
<td>11.62±0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PCV %</td>
<td>29.4±4.99</td>
<td>38.3±1.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total protein [g/dl]</td>
<td>5.88±1.12</td>
<td>7.03±0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose [mg/dl]</td>
<td>79.28±18.98</td>
<td>80.06±11.28</td>
<td>N.S</td>
</tr>
<tr>
<td>Urea [mg/dl]</td>
<td>30±5.30</td>
<td>29.4±4.84</td>
<td>N.S</td>
</tr>
<tr>
<td>Creatinine [mg/dl]</td>
<td>1.01±0.18</td>
<td>0.92±0.19</td>
<td>N.S</td>
</tr>
<tr>
<td>ALT [IU/l]</td>
<td>46.58±1.15</td>
<td>13.56±3.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST [IU/l]</td>
<td>13.76±1.76</td>
<td>13.2±1.88</td>
<td>N.S</td>
</tr>
<tr>
<td>MDA [μ mole/l]</td>
<td>2.59±0.26</td>
<td>1.52±0.23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vit. C [mg/dl]</td>
<td>2.07±0.16</td>
<td>2.11±0.18</td>
<td>N.S</td>
</tr>
</tbody>
</table>

As a result of the uncontrolled growth of leukemic cells in the bone marrow, there is an inadequate space in the bone marrow for normal blood production (hematopoiesis). The lack of normal blood growth results in the lack of normal white blood cells (increasing the risk of infection), the lack of red blood cells resulting in fatigue, weakness, and anemia ( low Hb and PCV) and also the lack of normal platelet production (increasing the risk of bleeding)[26]. Also early signs of ALL may be similar to those of the flu or other common diseases, such as a fever that won’t go away, feeling weak or tired all the time, aching bones or joints, or swollen lymph nodes. Common presenting symptoms including pale skin and weakness due to low hemoglobin levels (anemia)[27].It was found that total proteins will be lowered to because of the liver damage associated with ALL [29].

The level of MDA contents were shown to be increased in the just diagnosed patients, when compared with the control group[30]. These results may indicate a possible link between decreased activities of antioxidant enzymes and increased levels of oxidative damage, and support the notion that free radical reactions may be increased in malignant cells. Malondialdehyde (MDA) is the by product of free radical mediated reactions which lead to the formation of lipid peroxides, alcohol and sectionaldehydes. Normally, MDA is quickly oxidized to acetate or malonate and then to carbondioxide by the Kreb’s cycle. If it is accumulated in excess, MDA can combine with different serum proteins and cell membrane components to form altered determinants . It can interact with deoxyribonucleic acid (DNA) and inhibit the biosynthesis of the DNA, ribonucleic acid (RNA) and proteins. The chemical structure of MDA closely resembles that of carcinogenic compounds like glycidaldehyde and beta propiolactone and it thus may itself, have carcinogenic properties . Erythrocytic lipsids are more
susceptible to auto-oxidation under conditions of oxidative stress, more so, in the acute leukaemia patients. Erythrocytic MDA (eMDA) has been found to be increased in leukaemia. As MDA has the propensity to attack the sulfhydryl group, it may be involved in the alteration of the erythrocytic PK (Erythrocytic Pyruvate Kinase) levels in acute leukaemia patients. It has been reported that certain lipid peroxidation products like MDA can attack the sulfhydryl groups and the amino group in proteins. The sulfhydryl group of PK is prone to this action by MDA. Thus, increased eMDA may also be a causative factor for the decrease of the ePK activity in acute leukemia patients.[31]

In this study, a significantly negative association was observed between ALT [IU/l] with PCV % in Patients group (r= 0.22, p<0.05), while there was no significant correlation was observe in the control group as shown in figure 2.

Serum proteins are beneficial signs for initial screening of any abnormal function, inflammation and many disorder. The appearance of different proteins may differ depending on the age of the person [32]. The current study showed that there was a significant decrease in serum protein as shown in table 1, and agreement with other studies. [33, 34] Acute protein loss is commonly due to reduced protein consumption together with a hyper metabolic state resulting in rapid reduction of visceral proteins.

The recent study results conclude that high levels of ALT and MDA at the diagnosis may be due to the effect of induction therapy treatment an unfavorable consequence in ALL Iraqi child.

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دراسة مستويات بعض المؤشرات الكيمو- حيوية لدى الأطفال العراقيين المصابين بسرطان الدم اللمفاوي الحاد

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الخلاصة:
تعتبر اللوكيميا أو سرطان الدم من أكثر أنواع السرطانات شيوعاً في الطفولة. ويعتبر سرطان الدم اللمفاوي الحاد ALL من أكثر أنواع سرطان الدم حدوثاً لدى الأطفال. ويعتبر تطور عدد كبير من خلايا الدم البيضاء غير الناضجة في دم الطفل المصاب ونخاعه العظمي. كما ورص هذا المرض الرائعين أيضاً إلا أن علاج الأطفال يكون مختلفاً حيث يظهر على الأطفال إعراضاً متسببة من تسلل خلايا الدم البيضاء غير المتخصصة في نخاع العظم إلى الجهاز اللمفاوي والمواقع خارج نخاع الدم كالجهاز العصبي المركزي. من أكثر أعراض ALL شيوعاً والإعاقة (50%)، الشحوب (25%)، الحمى (60%)، وخسارة الوزن (26%)، كما أن تسلل ALL كريات الدم البيضاء غير المتخصصة إلى تجاويف نخاع العظم وعظام النخاع (السحاق) غالباً ما يؤدي إلى تأخير العظام (25%)، كما يسبب أعراض متعددة في نخاع الخلايا الدم الطبيعية، ويلاحظ أن 75% من المرضى يصابون بنقص في الصفيحات الدموية تتراوح أعمارهم بين 16 و1 سنة بعد شهر من بدء العلاج. ونقص متوسط Hb لدى 40% من الأطفال المصابين إلى أقل من 100,000 Hb /mm3، وانخفاض مستوى Hb لـ 7 من الحالات المرضية ارتفاع تعداد كريات الدم البيضاء إلى أكثر من 500,000 mm3، كما وجد أن نقص كريات الدم البيضاء المتخصصة (قلل من 5000 شائعة) وانتشار زيادة خطر الإصابة بالمرضى. هذه الدراسة تم التحقق من بعض المؤشرات في الدم والتي يمكن اعتبارها دلالة للإصابة بسرطان الدم اللمفاوي الحاد. في هذا الاعتقاد، تم دراسة مستويات الأحماض، البروتين، AST، ALT، AST، ALT، AST، ALT، AST، AST، ALT، AST، ALT، AST، ALT، AST، ALT، AST، ALT، AST، ALT، AST، ALT، AST، ALT، AST، ALT، ALT و بعض مضادات الأكسدة. جمعت النماذج من 50 مثلاً مرضي BCR-ABL، والذي تتراوح أعمارهم بين (1-16) سنة بعد شهر من تعاطيهم للعلاج. ووجدنا علاقة سلبية متعددة بين مجموعات مختلفة في إنزيم AST عند الدراسة مع مجموعة السرطان. كما ورصد وجود علاقة سلبية ما بين ALT و PCV لدى مجموعة المرضى (r = 0.22, p<0.05). و بيما لم يتم رصد علاقة ملحوظة في مجموعة الأصحاء.

الكلمات المفتاحية: سرطان الدم اللمفاوي الحاد، إنزيم AST، ALT، MDA.