Assessment of the Role of Oxidative Stress and Circulating Biochemical markers in Childhood Leukemia

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Abstract. Acute lymphoblastic leukemia (ALL) constitutes a family of genetically heterogeneous lymphoid neoplasms derived from B- and T-lymphoid progenitors. The aim of the study was to assess the oxidative status through measurement of antioxidants levels in patients with ALL and deduce any differences in their concentrations from normal values. The study was included 60 children with acute lymphoid leukemia diagnosed by blood film and bone marrow examination and 30 healthy subjects taken as healthy for the initial laboratory tests. Oxidative stress, blood antioxidants status, and liver enzymes profiles were measured in all groups. Our results showed that Antioxidants levels and Hb were significant decreased (P 0.05) in ALL patients. In contrast MDA, liver enzymes, leukocytes and Lymphocytes count were significant increase (P 0.05) in ALL patients compared to healthy control subjects. Boys predominance was evident, the fraction of patients living in urban areas was more than those of rural regions. Also, the results from statistical analysis revealed that there was a strong correlation between ABO blood groups and leukemia. The present study concludes that free radicals have been concerned in the pathogenesis of leukemia in childhood, the mechanism of increased MDA is that may be due to lymphocyte cells are source superoxide and other oxygen metabolites which lead to lipid peroxidation.

Keywords: ALL, Oxidative stress, Vitamins, GSH, Liver enzyme.

Introduction

Acute lymphoblastic leukemia (ALL) or childhood leukemia is a cancer of the blood and bone marrow [1] ALL is a disorder caused by an abnormal expression of genes which leads to many bone marrow stem cells develop into a type of white blood cell called lymphocytes [2] 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 [3] ALL is the most common type of cancer in children. About 80% of children with leukemia have acute lymphoblastic leukemia. 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 2-5 years of age [4] Although, the exact cause of leukemia is still unknown, scientists suspected that viral, genetic, environmental or immunological factors have been implicated in the pathogenesis of this cancer [5].
The generation of reactive oxygen species (ROS) and other free radicals during metabolism is a necessary and normal process that ideally is compensated for by an elaborate endogenous antioxidant system [6]. However, due to many environmental, lifestyle, and pathological situations, excess radicals can accumulate, resulting in oxidative stress [7]. Oxidative stress has been related to cardiovascular disease, cancer, and other chronic diseases that account for a major portion of deaths today [8]. Prime targets of free radicals are the polyunsaturated fatty acids in cell membranes causing lipid peroxidation which may lead to damage to the cell structure and function [6]. Additionally, decomposition of lipid peroxidation a wide change of end product including malondialdehyde (MDA) which is considered as a marker for oxidative stress through many investigators [9]. Under normal conditions, defense mechanisms of the body play an important role in the form of antioxidants and therefore, minimize the damage, adapting itself to the stressful situations [10]. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions and play a major role in the prevention of various diseases including cancer and their clinical manifestations [5].

The aim from this research is investigate the inter relationship among childhood leukemia with circulating biochemical markers and oxidative stress in the Iraq children.

**Material and Methods**

The study was done on 60 patients diagnosed with ALL and the records of the patients were studied in regards to their age, sex, blood group and residence. The control group consisted of 30 apparently healthy individual who were not complaining of any blood problem.

Venous blood samples five ml was drawn from sixty patients of ALL ranging between (1-17) years old. Blood samples have been collected into two vacationer tubes, one containing EDTA for measurement of blood hemoglobin (Hb), leukocytes and lymphocytes count. The blood in the second part was allowed to clot for 10-15 min. at room temperature, centrifuged for (10) min. at (3000rpm). Serum was removed for measuring of biochemical parameters.

The total serum MDA measured according to the modified method of Benge (Benge, 1978). Glutathione was estimated by the method of Beutler’s method (Beutler et al., 1963). Vitamin E, vitamin A and vitamin C levels were determined according to a modified of Pagana [11].

Liver enzymes test were measured using the standard kits from Biolabo. That include Gamma glutamyl transferase (GGT) [12], Aminotransferase enzymes (ALT, AST) and Creatine kinase (CK) [13], Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) [14].

In this study, all statistical analyses were performed using SPSS (Statistical Package for Social Science, Inc., USA) (version 17.0). Data were presented as mean ± SD. Association between ABO/Rh phenotypes of patients, gender and ALL was done using chi-square test. The probability $P \leq 0.05$ = significant, $P > 0.05$ = non-significant.

**Results**

There is no significant different ($p > 0.05$) age between leukemia patients group and healthy control subjects. There were significant decreases ($p < 0.05$) in Hb in patients when compared to healthy control subjects. While were found to be significantly increase ($p < 0.05$) in leukocytes and lymphocytes count in patients group when compared to healthy control subjects as shown in table 1.
Table 1. Age, leukocytes, lymphocytes count and hemoglobin concentration of different studied groups (Mean± SD).

| Characteristic | Healthy control subjects (n=30) Mean ± S.D | Patients Group (n=60) Mean ± S.D |
|----------------|--------------------------------------------|---------------------------------|
| Age            | 7.98 ± 3.43                                | 7.30 ± 3.84                     |
| Leukocytes *10³/µl | 6.64a ± 2.74                               | 11.04b ± 3.63                   |
| Lymphocytes *10³/µl | 1.76a ± 2.39                               | 5.03b ± 2.43                    |
| Hb(g/dL)       | 13.31a ± 1.34                              | 8.33b ± 0.62                    |

*Significant at 0.05 level of significance

The result showed that there were a significant increase (p 0.05) in the mean of MDA level of ALL patients group to compared with healthy control subjects, while there was a significant decrease in the mean concentration of serum antioxidant (vitamin C, vitamin E, vitamin A and Glutathione (GSH)) in patients group in comparison with mean serum antioxidant concentration of healthy control subjects as shown in table 2.

Table 2. The biochemical parameters of different studied groups (Mean± S.D).

| Characteristic | Healthy control subjects (n=30) Mean ± S.D | Patients Group (n=60) Mean ± S.D |
|----------------|--------------------------------------------|---------------------------------|
| MDA nmol/ml    | 3.15± 0.32                                 | 10.19± 0.24                     |
| Vitamin C mg/dl| 2.15± 0.14                                 | 1.85± 0.19                      |
| Vitamin E µmol/L | 23.83± 1.08                               | 9.25± 0.95                      |
| Vitamin A µg/dl| 26.37± 0.98                                | 12.25± 1.23                     |
| GSH mg/dl      | 9.23± 0.44                                 | 2.42± 0.36                      |

*Significant at ≤0.05 level of significance

The results showed a significant increase (p 0.05) in liver enzymes activity (GGT, ALP, ALT, AST, LDH, CK) in patients group when compared with healthy control subjects (figure 1).

Figure 1. The activity of liver enzymes in ALL patients and healthy subjects group.
The results showed that the ABO blood group frequencies occurred in the following order O > A > B > AB, respectively, among the overall individuals sampled. O blood group has the highest overall percentage frequency. AB blood group has the least overall percentage frequency. This was not the case with the healthy control subjects where the most common blood group was blood group B, followed by A, blood type AB came the third in frequency in the control group, then O blood type was the least common type in the healthy control subjects. As regards the Rhesus blood group system, we found that of the sampled population were Rh+ve while were Rh-ve. Also so is the case with healthy control group as shown figure 1.

![Figure 2. Relationship between ABO/Rh phenotypes of ALL patients and the healthy control subjects.](image)

**Discussion**

The results in table 1 showed increase in leukocytes count in patients when compared with healthy control subjects, but in spite of the high count of WBC, there are shortage of normal forms i.e. since childhood leukemia like all cancers is a product of two or more molecular changes in stem like cells that have the ability to divide while maintaining an immature state [15].

In this study, lymphocytes count was significantly higher in patients with ALL than healthy control subjects. This result agreed with study of [3]. This can be explained by the fact that in leukemia there is a clonal proliferation of malignant cells that may arise at any stage of maturation in the bone marrow including lymphoid, myeloid or pluripotent stages [14].

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 decreased in ALL patients [16]. These studies are harmony with the results of the present study (Table 1) which showed that Hb in acute lymphoblastic leukemia patients was significantly decreased as compared to control healthy subjects, this indicate there is correlation between anemic ALL patients and decrease of some antioxidants level [17]. Hb was found statistically significant to be decreased in ALL patients compared to controls. This finding may indicate a possible link between decreased Hb and
decreased levels of GSH result to oxidative damage, supporting the idea that there is a persistence of oxidative stress leading to cellular dysfunction and cell death. Accumulation of such molecules causes noxious effects on individuals, resulting in diseases such as hematopoietic malignancies such as acute lymphoblastic leukemia [18].

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) [18]. Data in the table 2 showed there is significant increase in the mean concentration of serum MDA of ALL patients group in comparison with mean serum MDA concentration of control group these result is similar to previous study presented by [19]. That result is shows a significant increased lipid peroxidation in leukemia patients possibly, as a result of oxidative stress due to free radical production and that approved with other study [6].

The serum MDA levels act as an important biomarker of leukemia having diagnostic and prognostic role indicating disease progression [20]. The results in the table 2, reveal a decrease in the levels of vitamins A, E and C in ALL patients. Such decreases may play a great role in the development of malignancy, since that antioxidant vitamins reveal great actions in the physical and chemical quenching of free radicals generated from oxidation processes inside the human cell [21]. Vitamin A was shown to be involved in the stimulation of the immune system and cancer suppressor genes as well as deregulate of oncogenes and block tumorigenesis [22]. Vitamin E was reported to be an important factor in the induction of apoptosis of cancer cells beside its action in quenching of free radicals and increase of the capability of the immune system [2]. Vitamin C is considered the most powerful natural antioxidant, which protects indispensable macromolecule in the human body from damage by free radicals [22]. The one main reason behind the decrease in levels of antioxidant vitamins the malnutrition because the under-nourished persons have impaired immune and hematopoietic systems [23]. GSH is involved in DNA synthesis and repair. In case of leukemia reduced levels of GSH reflects the depletion of non-enzymatic antioxidant reserve. It reflects that the GSH depletion, which normally acts as an antioxidant factor [24]. Li [25] mentioned that GSH deficiency may indeed responsible for the immunological non-responsiveness under the excessive antigenic stimulation by non-professional stimulator cells. Reduced GSH is an important key factor have important role as scavenger of free radicals.

The correlation analysis showed that there was a negatively correlated between MDA with GSH, Vit.C, Vit.A and Vit.E (r= -0.72, -0.63, -0.61, -0.65, -0.59 respectively, p  0.05). The effect of free radicals is balanced by enzymatic and non-enzymatic antioxidant. The impairment antioxidant role of vitamin A, C and E and GSH may support the accumulation of free radicals. Alternatively, it is possible that the antioxidant system is impaired as a consequence of an abnormality in the antioxidants metabolism due to the cancer process. This effect could be enhanced by the characteristic increase in the production of free radicals by the cancer cells and thus increase in MDA levels [26].

The results were shown in the figure 1. Increase in liver enzymes activity may be due to the hepatic dysfunction which is characterized by alterations in serum levels of liver enzymes. This abnormality may be due to alteration of the synthesis of these enzymes within the liver or a change in the permeability of the cell membrane brought about by the disease condition or due to a decrease in the rate of removal of these enzymes from the bloodstream [27]. All the disorders suggested that liver and hematological systems might be the target effectors of toxic effect induced by free radicals such as increase in liver enzymes and decrease in hepatic glutathione (GSH) [28]. Both the Chi square test was used to assess the relationship between the ABO/Rh phenotypes in the study patients. The obtained results showed a
significant association between ABO/Rh blood groups and ALL. The distribution of ABO/Rh phenotypes of patients was as in figure 2.

In the current study, blood group O stands firstly among the most common blood groups in the studied patients may be due to genetic predisposition for O blood group between types of blood groups for injury leukemia, or due to an environmental factor is assumed to be responsible for the high frequency of O blood group in patients, this in agreement with the study by [29] study in Saudi Arabia.

ABO blood groups are still fruitful research strategy in understanding the etiology of hematological malignancies. ABO blood groups have also association with other diseases like hepatitis and gastric carcinoma [28]. Another study was conducted in Iraq during 2008-2010 and it was found that 41.8% of leukemia patients have blood group O while 28.8% have B [30].

Childhood leukemia occurred slightly more frequently in boys than girls. This result agree with the result of [31]. The reason which may be assisting the male to infect of leukemia could be due to presence of the sex responsive gene near gene BCR-ABL which is located on a Philadelphia chromosome [20]. A study conducted in Baghdad Teaching Hospital showed dominance more male than female may be due to the nature of their jobs and hormones [32]. These results indicate that children living in urban areas experience an excess leukemia risk. This increased risk was often attributed to high exposure to atmospheric benzene and other pollutants from motor vehicular traffic, or to the nearby presence of industrial plants, waste incinerators, or other sources releasing potentially carcinogenic chemicals [33]. Other authors suggested that the excess risk found in urban areas was due to the spread of infectious agents through interpersonal contact, which is clearly more intense in urban areas where population density is higher and the proportion of commuters or immigrant residents is higher, with an increased degree of population mixing [34].

Conclusion

The results may indicate a possible link between decreased activities of antioxidant and increased levels of oxidative damage, and support the notion that free radical reactions may be increased in the malignant cells.

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