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Consideration of Liver Function test with Erythropoietin Hormone Levels in Iraqi Patients Diagnosed with Lymphoid and Myeloid Leukemia

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Abstract:

The study was carried out to measure the erythropoietin hormone level and a number of the hematological variables (red blood cells, platelets, monocytes and lymphocytes) and biochemical variables (erythropoietin, alkaline phosphatase, alanine transaminase and aspartate transaminase) for two types of leukemia (lymphoid leukemia and myeloid leukemia). Enzyme Linked Immune Sorbent Assay (ELISA) was used to detect level of erythropoietin in patients with lymphoid and myeloid, leukemia and control group , liver enzymes activity were measured including alkaline phosphatase (ALP) alanine transaminase (ALT) and aspartate transaminase (AST). Also estimated some hematological variables were estimated: red blood cells, platelets, lymphocytes and monocytes, as well as, ABO blood groups in all studied groups. The statistical analysis showed significant ($P< 0.05$) increase in the level of erythropoietin, liver enzymes, lymphocytes and monocytes number in patients with lymphoid and myeloid leukemia when compared with the control group. While, there was a decrease ($P< 0.05$) in red blood cells count and platelets count in the patients groups compared with the healthy group. Also, the results from statistical analysis revealed that there was a strong correlation between ABO blood groups and leukemia.

Keywords: *Erythropoietin, Leukemia, ALP, ALT, AST, RBC.*

Introduction:

Erythropoietin (EPO) is a glycoprotein consisting of 165 amino acids and four carbohydrate groups, and it has a molecule weight 30.4 Kd with a half-life of 6-9 hours (1). In the human body, EPO is produced primarily by endothelial cells of peritubular capillaries within the cortex of kidney at rate 90% and 10% of EPO is produced from the liver (2). It is worth mentioning, that the EPO production is regulated by the feedback mechanism including oxygen delivery to the different tissues (3). The hypoxia-inducible factor (HIF) is regulates the transcription of EPO gene in kidney, which determines EPO production, this process is dependent on the local oxygen tension (4). Regulation of EPO gene expression occurs essentially at transcriptional level by DNA-dependent on mRNA synthesis and the gene activation (5). EPO is the most important factor for red blood cells (RBC) production by activating the progenitor cells in bone marrow (6).

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Leukemia is one of the most common cancer types prevalent worldwide in both genders and all races regardless of the level of living. In Iraq, the statistics and epidemiological studies show that leukemia is a second most common cancer after breast cancer. It affects males 57% more than females 43% (7). For children, leukemia is most common type of malignancy and represent 34.97% of all childhood cancers (8). Leukemia affects blood-forming tissues especially the bone marrow, so, the patients infected with lymphoid leukemia and myeloid leukemia suffering from anemia which caused by bone marrow failure responsible for producing blood components (red blood cells, white blood cells, platelets), that stimulate their production by erythropoietin hormone, therefore the study was aimed to estimate EPO level in Iraqi patients diagnosed with lymphoid and myeloid leukemia.

Materials and methods:

1-Sample collection and grouping

This study was carried out at the department of Hematology\ Al-Yarmuk Teaching Hospital and Medical City in Baghdad\

Iraq, during the period from August 2016 to February 2017. During the period was collected seventy samples from Iraqi patients 37 infected with lymphoid leukemia and 33 infected with myeloid leukemia, their ages ranged from 6 to 68 years. Thirty-seven healthy persons (males and females) were taken as a control group with age range (7-63 years) and all participants consented to the study.

2- Blood Sample:

peripheral venous blood sample were collected from 70 patients was diagnosed with leukemia and 37 healthy donors as control group (consent was given from patients and healthy). Each sample was divided into two parts. The first part of blood was placed into standard hematological EDTA tubes to be used for hematological tests. The second part of blood was placed in a gel tube, whereby the serum was separated, and then incubated at a temperature of -20°C until the time of undergoing a biochemical tests.

3- Hematological tests:

The hematological tests encompassed red blood cells (RBC) count and platelets were measured according to the method which reported by Lewis et al., (2001) (9). The different white blood cells count including lymphocytes count and monocytes count were estimated via using the blood smear and stained by leishman stain (9) . Finally the ABO blood groups determined for every patient by method reported by Dacie and Lewis, (2005) (10) .

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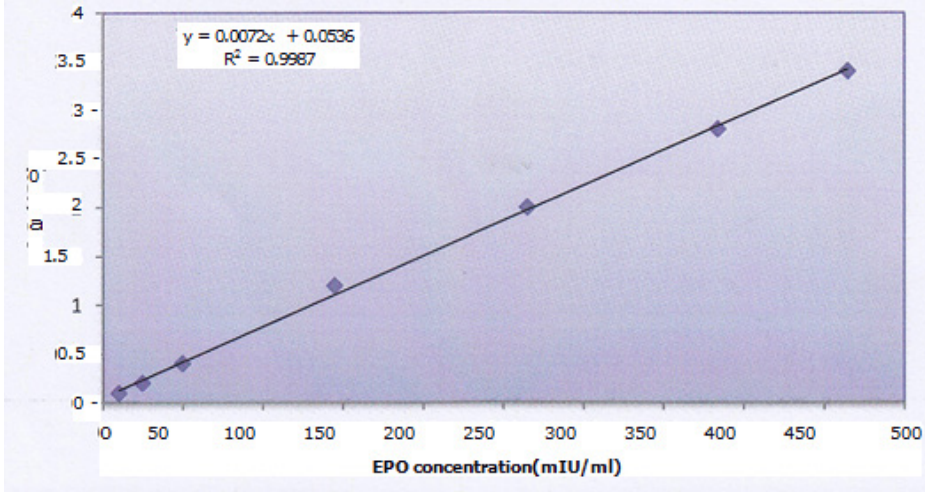

Figure 1: Representation Standard curve of hormone erythropoietin ELISA

B.Estimate some liver functions including Alkaline phosphates was measured by using spectrophotometric and this method reported by Belfield and Gold Berg, (1971) (11). On the other hand, transaminase enzymes were measured by using the directed kit method supplied by the Randox Engeland described by Reitman and Frankel, (1957) (12).

5- Statistical analysis:

Data were analyzed using Statistical Package for Social Science (SPSS, Version 17.0). The mean of continuous variables and frequency distribution of categorical variables were calculated.

Mean hematological and seriological variables were compared between study groups across the ANOVA statistical test. Blood groups distribution were compared among groups by using Chi-square. P-values ($P<0.05$) were considered statistically significant.

Results:

The current study showed that serum EPO concentration in patients with lymphoid and myeloid leukemia was significantly ($P<0.05$) increased (119.66 ± 28.63; 117.02 28.63 mlU/ ml respectively) than its level in control group (26.56 ± 26.12 m1U/m1) as demonstrated in the figure (1).

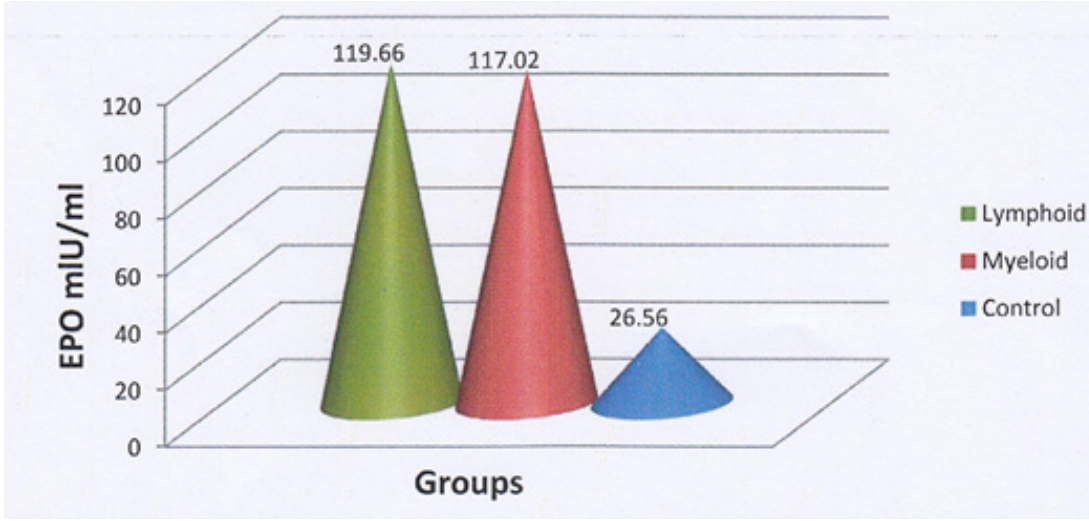

Figure 2: Level of hormone EPO concentration in serum of patients with lymphoid and myeloid leukemia compared to control group.

There was no significant difference in serum EPO concentration between types of leukemia (lymphoid and myeloid).

This study showed that liver enzyme activity was high in patients when compared with control group as shown in the table (1). The results of ALP showed a significant (P<0.05) increased in patients with lymphoid and myeloid leukemia (89.351 + 4.787; 95.754 ± 4.786 1U/L, respectively) when compared with the control subjects (69.840 ± 4.3671U/L). Also, our findings showed that the levels of ALT increased significantly (P<0.05) in lymphoid and myeloid patients (23.272 ± 2.379; 26.694 ± 2.3781 U/L, respectively) compared with the control group (19.502 ± 2.170 1U/L), and the values of AST in patients with lymphoid and myeloid leukemia (P5_0.05) (32.689 ± 1.983; 32.971 ± 1.983 lU/L respectively) were higher than control group (27.109 ± 1.809 lU/L).

Table -1: The liver enzymes activity tested (ALP, ALT, AST) in the different studied groups (patients and control).

Liver enzymes	Groups	Mean ± S.D
ALP lU/L	Control	69.84[a]± 4.36
	Lymphoid	89.35[b] ± 4.78
	Myeloid	95.75[c] ± 4.78
ALT lU/L	Control	19.50a ± 2.17
	Lymphoid	23.27[b] ± 2.37
	Myeloid	26.69b ± 2.37
AST lU/L	Control	27.10a ± 1.80
	Lymphoid	32.68[b] ± 1.98
	Myeloid	32.97[b] ± 1.98

ALP = lU/L, ALT = lU/L, AST = lU/L, P Value = P≤ 0.05. Different letters (a, b) means significant difference at P≤ 0.05 level.

The results of hematological variables showed that the levels of lymphocytes were significantly elevated (P< 0.05) in lymphoid leukemia patients (4.73 + 0.43*103/p1) compared with myeloid leukemia patients and the control group (3.15 + 0.43; 2.46 ± 0.39* 103/µl respectively), whereas the findings of monocytes count showed a higher significance (P<0.05) in patients with myeloid leukemia (3.11 + 0.22* 103/µl) when compared with lymphoid leukemia patients and the control group (2.06 + 0.22; 0.54 ± 0.50* 103/µl respectively). Red blood cells count were significantly decreased (P<0.05) in lymphoid and myeloid leukemia patients (2.90 ± 0.23; 3.30 + 0.23*106/Ial respectively) compared with the control group (4.78 ± 0.21*1064t1). The results of platelets levels showed a low significance (P>0.05) in patients with lymphoid and myeloid leukemia (135.11 + 14.34; 167.63 ± 15.34*103/p1 respectively), when compared with the control .group (200.94 + 13.998* 103/µl) as shown in the table (2).

Table -2: The hematological variables in the different studied groups.

Hematological variables	Groups	Mean ± S.D
RBC ***10□/µl**	Control	4.78[a] ± 0.21
	Lymphoid	2.90[b] ± 0.23
	Myeloid	3.30[b] ± 0.23
Platelets ***10³/µl**	Control	200.94[a] ± 13.99
	Lymphoid	135.11[b] ± 15.34
	Myeloid	167.63c ± 15.34
Lymphocytes ***10³/µl**	Control	2.46a ± 0.39
	Lymphoid	4.732b ± 0.43
	Myeloid	3.15c ± 0.43
Monocytes ***10³/µl**	Control	0.54[a] ± 0.20
	Lymphoid	2.06b ± 0.22
	Myeloid	3.11c ± 0.22

RBC = *10□/µl, Platelets = *103/µl, Lymphocytes = *103/µl, Monocytes = *103/µl, P Value = P≤ 0.05

The results of the statistical analysis did not show any significant differences in the biochemical parameters and hematological variables between the subtypes of leukemia (lymphoid and myeloid) (P<0.05).

Then distribution of blood types at patients infected with leukemia and control group was tested during the period that has been mentioned previously. Blood group B was more distributed among the other types of ABO blood groups. Moreover, the blood group A was less frequent in patients suffering from leukemia, following by blood group 0, lastly AB as shown in the figure (3).

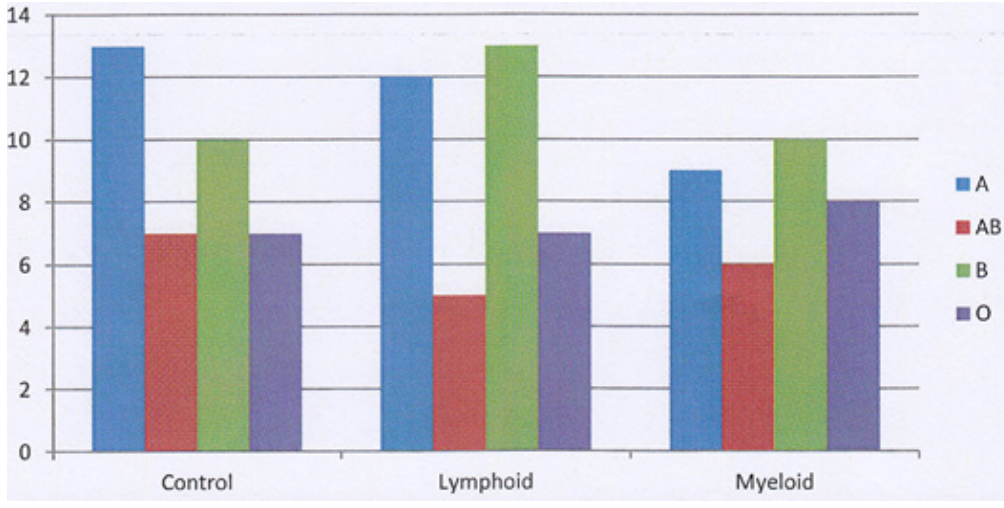

Figure 3: Distribution of lymphoid, myeloid leukemia and control groups according to ABO blood system.

Discussion:

This study give anew considerable ideas about serum EPO levels in patients with lymphoid and myeloid leukemia compared to healthy (control group) in Iraqi samples. This finding also was detected in the study of Molica et al., (2011) (13).

Leukemia was characterized by abnormal an accumulation of immature white blood cells called blasts which have the ability to interfere with the formation of the natural blood components by the bone marrow, this leads to the erythrocytopenia, leukocytopenia, thrombocytopenia, often cause many symptoms due to the suppression of natural blood components and consequent anemia (14) which might be stimulate a higher increase in EPO production in kidney, thereby leading to a significant increase of hormone concentration in blood serum (15). This in turn, can be the major cause for the obtained result. Another possible cause is that the EPO is considered as angiogenic agent (16).

The levels in the liver enzymes activity may be due either to direct infiltration of leukemia cells into the liver as a result of a defect in the membrane of mitochondria and cytoplasm or a damage in the liver tissue due to a certain immune response. The present results consistent with those of previous studies by Bdaiwi, (17) and Lammers et al., (18). However, the results not consistent with the findings by Lee, et al., (19) which showed that the damaged liver cells fail to produce ALP.

A previous study by Bukowcz et al., (20) established that the ALP is a different isoenzyme mostly excreted from the hepatic tissue and bone tissue into the bloodstream. The another reported that high activity of this enzyme is considered as a sign of this tissue damage. Also, Nishizawa et al., (21)

observed that the bone formation markers are materials directly or indirectly formed via osteoblasts at each stage of the osteoblast differentiation, They reflect different aspects of the osteoblast function and bone formation. We can measure most of the substance in blood serum by ALP which is considered as an indicator. A pervious study by Heaney et al., (22) reported that the phosphate is a very important mineral and it is necessary for all cells in the body in order to perform their natural functions.

With respect to the ALT and AST activity in the blood serum, the results of the study showed a significant increase in ALT and AST activity in patients with lymphoid and myeloid leukemia when compared with the control group. This outcome was in consistent with previous studies that showed a significant increase in these enzymes in blood serum of patients suffering from leukemia, liver cirrhosis and viral hepatitis (23). Another study by Langan and Zawistoski, (24) mentioned that in the case of anemia, it is normal to find a deficiency in vitamin B12 thereby the liver tries to capture the largest quantity of this vitamin. Hence temporary hypertrophy occurs in the liver and this is reflected on the effectiveness of these enzymes.

The study showed a significant reduction of red blood cells also due to anemia which characterized by a decrease in red blood cells count and concentration of hemoglobin in blood. Anemia is one indicators for infected leukemia diagnosis, in addition, it is a distinctive feature of bone marrow disorders. This finding is consistent with study by Michallet et al., (25). A statistically significant decrease in platelets number was found in patients with lymphoid and myeloid leukemia compared with the control group in the present study. This decrease in platelets count could be due to the expansion of abnormal blasts cells in bone marrow thus leading to failure in the bone marrow which produce normal blood elements (26). The researcher Jemal et al., (27) confirmed that the pancytopenia in leukemia patients due to the physical replacement of the bone marrow cells by immature blasts and secretion of the inhibitory agents by abnormal white blood cells and thus affect in the normal hematopoiesis.

The results of the statistical analysis showed a significant increase in lymphocytes count in patients with lymphoid leukemia. This decrease may be due to a somatic mutation in lymphoid progenitor cells which change the regulation of cellular proliferation, differentiation and apoptosis (Program cell death), and spread an accumulating of the lymphocytes in lymph nodes and thus causing lymphadenopathy (28) . While, the results of study showed increase in monocytes count in patients. This is not astonishing in patients with myeloid leukemia when compared with other study groups because in patients suffering myeloid leukemia a malignant change begins in myeloid stem cells which form red blood cells. Some types of white blood cells and platelets therefore occur leading to an increase in the number of these cells. The results are in agreement with that in a study by Mallouh et al., (29).

The results showed that the blood group B is more frequent in patients with leukemia, where previous studies have shown that there are at least two influences working the spread of leukemia in Iraq. First is could be due to the genetic predisposition of B blood group among other types of blood groups and leukemia, also the environmental agents are assumed to be responsible for the high frequency of B blood group in patients with leukemia. The findings of study by Hasanein et al., (30) conducted in Baghdad support the findings. The other explanation which is by Keita, is the expansion of abnormal blasts cells in bone marrow thus leading to failure in the bone marrow which produce normal blood elements (26). The researcher Jemal et al., (27) confirmed that the pancytopenia in leukemia patients due to the physical replacement of the bone marrow cells by immature blasts and secretion of the inhibitory agents by abnormal white blood cells and thus affect in the normal hematopoiesis.

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