

# Evaluating The Levels Of Vitamins E, C, Malondialdehyde And Some Hematological Parameters And Gammaglutamyl Transferase In Patients With Leukemia

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## ABSTRACT

Leukemia is one of the most common cancer prevalent worldwide in both gender, and all races regardless of the level of living. It affects blood-forming tissues especially the bone marrow. Due to the importance of this disease and its risks, the study was carried out to evaluate the oxidative stress status and detect the effect of leukemia on some hematological parameters. Estimate gamma glutamyl transferase (GGT).

A study was conducted between August 2016 till February 2017 in the center for Hematology/ Yarmouk Hospital. The blood samples were collected from 70 Iraqi patients with leukemia and 37 normal persons as control group, their ages ranged from (6-68 years).

The statistical analysis of malondialdehyde (MDA) showed significant increase ( $P \leq 0.05$ ) in patients with leukemia in compared to healthy. While there was significant decrease in Vitamin C and E concentration in patients ( $7.10 \pm 0.49$ ;  $8.75 \pm 0.65 \mu\text{mol/L}$  respectively) in compared with healthy group ( $18.83 \pm 0.67$ ;  $25.83 \pm 0.88 \mu\text{mol/L}$  respectively). The results of gamma glutamyl transferase (GGT) showed significant increase ( $P \leq 0.05$ ) in patients with leukemia compared to healthy group. The results of hematological parameters showed significant decrease ( $P \leq 0.05$ ) in each of packed cell volume (PCV) in patients with leukemia compared to healthy group, and hemoglobin concentration in patients compared to healthy group, while there was a significant increase ( $P \leq 0.05$ ) in total leukocytes count in patients with leukemia when compared to healthy group. The results of the study showed a positive correlation between GGT and WBC.

## Introduction

Leukemia is one form of cancer which affect hematopoietic tissue and thus the bone marrow produces abnormal white blood cells have a growth advantage over the natural cellular elements due to an increased rate of proliferation against the lowering rate result bone marrow failure <sup>(1)</sup> . These abnormal cells called blasts cells which cause numerous symptoms due to bone marrow failure and infiltration of organs such as lymph nodes, liver, skin, kidney, spleen, muscles and others <sup>(2)</sup> . Four main kinds of leukemia

are: acute lymphoid leukemia (ALL) is the most common kind of leukemia in young children<sup>(3)</sup>, acute myeloid leukemia (AML), chronic lymphoid leukemia (CLL) and chronic myeloid leukemia (CML) <sup>(4)</sup>. Oxidative stress is a well-established general mechanism for cells and tissues injury <sup>(5)</sup>, oxidative damage is caused mainly by free radicals which are a chemical molecules possess an unpaired electron in the outer shell so are unstable thereby react quickly with other compounds to try to capture an needed electron to gain the stability <sup>(6)</sup> . The antioxidant defenses are very important as they represent a direct removal for free radicals, also providing maximal protection for the biological sites <sup>(7)</sup> . The main targets of free radicals

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are a polyunsaturated fatty acids in the cell membranes causing lipid peroxidation, that may lead to damage in the cell functions and structure <sup>(8)</sup>. Moreover, decomposition of lipid produce a wide variety of end products such as malondialdehyde (MDA) which is considered as a sign of oxidative stress. There are many evidence that has shown the importance and role of the antioxidant vitamins in prevention and treatment of cancer <sup>(9)</sup>. Vitamins has known to play an important role in prevent the cell damage and keep immune system against the damage caused by free radicals <sup>(10)</sup>. Vitamin C is one of most important and essential vitamins for the individual health, many health benefits have been attributed to vitamin C is a namely antioxidant, anti-atherogenic and the anti-carcinogenic activity <sup>(11)</sup>.

This study was aimed to investigate the relationship between leukemia and oxidative stress status through determination of level of MDA, vitamin E, C, gamma glutamyl transferase and some hematological parameters.

#### **Material and methods**

The study was done on seventy patients diagnosed with leukemia (37 patients with lymphoid leukemia and 33 patients with myeloid leukemia), between August 2016 to February 2017, at the Department of Hematology\ Al-Yarmuk Teaching Hospital and Medical City in Iraq. Thirty-seven healthy subjects (males and females) were taken as a control group with age range (7-63 years) and all participants consented to the study.

Six ml of blood samples were collected and divided into two tubes. The first part: 2ml of blood put in first EDTA tubes for measurement some hematological parameters. Either a second part: 4ml of blood was left for 20 minutes at room temperature, and after blood coagulation, the serum were separated by centrifugation at 3000 rpm for 15 minutes, then serum was removed for measuring of the biochemical parameters. The concentration of vitamin C in the collected serum samples was determined according to the modified method <sup>(12)</sup>. Standard curve was established for vitamin C determination using authentic samples of the vitamin. The concentration of vitamin E in the collected blood serum samples was estimated according to modified method <sup>(13)</sup>. Serum

MDA was measured by using test kit supplied by the Biolabo French <sup>(14)</sup>. Gamma glutamyl transferase (GGT) activity was also estimated by the test kit <sup>(15)</sup>. As for the blood variables including: blood hemoglobin (Hb) concentration it has been measured according to methods <sup>(16)</sup>, PCV was measured according to method <sup>(17)</sup>, total WBC measured according to a modified method <sup>(18)</sup>.

#### **Statistical analysis**

All statistical analyses in present study were done by 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 Biochemical variables were compared between study groups across the t-test. Sex were compared among groups by using Chi-square. The probability was  $P \leq 0.05$  consider as significant.

#### **Results**

The results of Vitamin C concentration showed significant decrease ( $P \leq 0.05$ ) in patients with leukemia ( $7.10 \pm 0.49 \mu\text{mol/L}$ ) in compared to healthy group ( $18.83 \pm 0.67 \mu\text{mol/L}$ ). As well as, the statistical analysis showed significant decrease ( $P \leq 0.05$ ) in vitamin E concentration in serum blood of patients ( $8.75 \pm 0.65 \mu\text{mol/L}$ ) compared to healthy group ( $25.83 \pm 0.88 \mu\text{mol/L}$ ).

A statistically insignificant higher significant increase ( $p \leq 0.05$ ) of MDA concentration in patients with leukemia ( $25.07 \pm 1.32 \text{nmol/ml}$ ) compared to healthy group ( $4.17 \pm 1.21 \text{nmol/ml}$ ). Mean Hb was significantly lower in patients with leukemia ( $8.33 \pm 0.28 \text{g/dl}$ ) compared to healthy group ( $13.31 \pm 0.38 \text{g/dl}$ ). The results of PCV showed significant decrease ( $p \leq 0.05$ ) in leukemia patients ( $23.53 \pm 0.64 \%$ ) compared to healthy group ( $40.61 \pm 0.87 \%$ ). The results showed a significant increase ( $P \leq 0.05$ ) in total leukocytes count in patients ( $14.41 \pm 0.51 * 10^3 / \mu\text{l}$ ) compared to healthy group ( $7.02 \pm 0.96 * 10^3 / \mu\text{l}$ ). A significant increase in serum GGT activity in patients with leukemia ( $19.05 \pm 0.8 \text{IU/L}$ ) compared to healthy group ( $12.41 \pm 1.08 \text{IU/L}$ ).

**Table 1. Biochemical and hematological parameters of different studied groups.**

parameters		Mean ± S.D
Vitamin C μmol/L	Control	18.83 <sup>a</sup> ± 0.67
	Patient	7.10 <sup>b</sup> ± 0.49
Vitamin E μmol/L	Control	25.83 <sup>a</sup> ± 0.88
	Patient	8.75 <sup>b</sup> ± 0.65
MDA nmol/ml	Control	4.17 <sup>a</sup> ± 1.21
	patients	25.07 <sup>b</sup> ± 1.32
Hb g/dl	Control	13.31 <sup>a</sup> ± 0.38
	Patient	8.33 <sup>b</sup> ± 0.28
P C V %	Control	40.61 <sup>a</sup> ± 0.87
	Patient	23.53 <sup>b</sup> ± 0.64
WBC *10 <sup>3</sup> /μl	Control	7.02 <sup>a</sup> ± 0.69
	Patient	14.41 <sup>b</sup> ± 0.51
GGT IU/L	Control	12.41 <sup>a</sup> ± 1.08
	Patient	19.05 <sup>b</sup> ± 0.80

\*Significant at 0.05 level of significance. S.D =Standard Deviation. Vitamin C = μmol/L. Vitamin E = μmol/L. Hb =g/dl. P C V =%. WBC =\*10<sup>3</sup>/μl. GGT = IU/L.

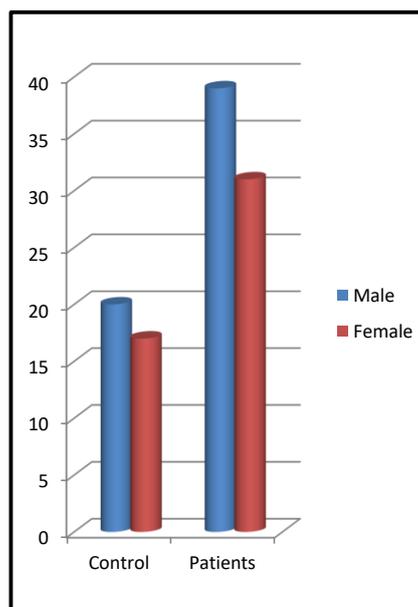
Through the present study was used a multiple regression analysis for study and predict the relationship between a variable WBC and other variables dependent on it. The results show that no quantitative relationship between the variables dependent on variable WBC expect GGT showed strong positive correlation with WBC, and it is considered important indicator of the disease, according to the linear equation:

$$Y = \alpha x + b$$

$$Y = 7.505 x + 0.252$$

$$WBC = 7.505 (GGT) + 0.252.$$

Sex distribution were compared among groups, the results showed that the males were more susceptible to leukemia than females in patients group, as well as in control group as showed in figure 2.



**Figure 1. Distribution studied according to sex.**

## Discussion

There are several indications which established the role of reactive oxygen species (ROS) and free radicals in initiation and promotion of different types of cancers such as leukemia <sup>(19)</sup>. Also, that the incidence of leukemia increasing with advancing age due to increasing level of free radicals reaction with age and to the decreased ability of immune system to detoxify those free radicals <sup>(20), (21)</sup>. Many of disease like Parkinson disease, atherosclerosis, kidney disease, diabetes, fibrosis, amyotrophic lateral sclerosis and aging and other pathological events might also due to enzymatic and non-enzymatic oxidation of the biological molecules <sup>(22)</sup>.

Many of enzymes, vitamins, trace elements and organic molecules play a main role in scavenging those free radicals through abandoning their electrons to free radicals in order to become more stable and which normally generated from oxidation processes inside human cells <sup>(23)</sup>.

The results of statistical analysis as shown in table (1) revealed a decrease in levels of vitamin C and vitamin E in leukemia patients. Such decrease might

played a great role in development of malignancy because that the vitamins reveal great actions in physical and chemical quenching of oxygen and superoxide radicals which generated from the oxidation processes inside human cells <sup>(24)</sup>. The low level of vitamin C attribute the large amounts of abnormal WBC which are working to abduct and remove a remaining of vitamin C <sup>(25)</sup>. In the case decreased concentration of this vitamin in serum blood the collagen production will stop and a mechanical strength of tissues, blood vessels and capillaries will weaken and the hemorrhage will result. Moreover many health benefits have been attributed to vitamin C namely antioxidant, anti-carcinogenic activity and anti-atherogenic <sup>(26) (27)</sup>.

Mehde, *et al* (2014) <sup>(28)</sup> mentioned that the Vitamin E was an important agent in induction of apoptosis of leukemia cells add to its work in reducing of the free radicals and increase of the capability of immune system. Vitamin E is considered one of the most important natural antioxidant and indispensable macromolecule to protect the cells human body from damage by free radicals <sup>(29)</sup>.

The decrease in serum concentration of vitamins was greater in Iraqi patients with leukemia in comparison with the western population, this might attributed to the nutritional differences between different societies and to a difference in the levels of water, air and food pollutants among those populations <sup>(30)</sup>.

The data from statistical analysis in the table 1 showed there was high significant increase in mean concentration of serum MDA of patients with leukemia in comparison with the mean serum MDA concentration of healthy group ( $p < 0.05$ ) these results were accordance with previous study presented Zainulabdeen, (2014) <sup>(31)</sup> which showed that the high increased lipid peroxidation in patients with leukemia might as a results of the oxidative stress due to free radical production.

The mean of PCV level in the present study was lower in patients with leukemia than control group. This decrease of PCV might be due to the special characteristics of pathogenesis of leukemia as it is started through a clonal proliferation of lymphoid or myeloid precursors depending on the type of

leukemia with a reduced ability to the differentiate into mature cellular elements <sup>(32)</sup>, as a result there was the accumulation of leukemia blasts or abnormal forms in bone marrow and peripheral blood with a variable decrease in the synthesis of natural red blood cells <sup>(33)</sup>.

The results showed a significant decrease in the concentration of Hb in patients with leukemia comparison with mean Hb concentration of healthy group, this result is consistent with the outcome by Veena, *et al.* (2012) <sup>(34)</sup> mentioned the anemia has characterized by a reduction of the each following: Red blood cell (RBC) count, the concentration of hemoglobin in the blood. Anemia is one features for leukemia diagnosis, it is a prevalent manifestation of bone marrow disorders, memorable should consider of the past medical history for the patients, and the family history <sup>(35)</sup>, It is necessary to conduct a physical examination especially splenomegaly where consider is the most important physical result in anemia patient. Lastly when upon analyzing the above information, one should estimate whether the mainly cause of anemia is the failure of bone marrow to form sufficient number of RBC or the accelerated loss <sup>(28)</sup>.

In the present study, findings of WBC count was significantly higher in patients as compared to healthy control. This result could explained according to Wang, *et al* (2011) <sup>(36)</sup> through a fact that in leukemia there is an increase in clonal proliferation of leukemia cells which may arise in any stage of the maturation in bone marrow as lymphoid, myeloid or a pluripotent stages.

The findings had reported that the significant increase in GGT activity in patients might attributed to continuous release of GGT from the surface of leukemia cells and its direct leakage to blood circulation. It is worth mentioning that the GGT is a membrane-bound enzyme, and which is affected significantly in the cancer cell more than the natural cell due to the changes of cell membrane accompanying the carcinogenesis, so this enzyme considered as marker to tumor progression and can be used for the monitoring of malignant tumor regression. our results are consistent with numerous studies by Liu *et al.* (2008) <sup>(37)</sup> and Kream, (2015) <sup>(38)</sup>.

This correlation could explained on the basis of a fixed fact is that this enzyme is closely associated to

the cell membrane and it affected significantly in cancerous cell more than the normal cell due to changes accompanying the carcinogenesis<sup>(39)</sup> and that occur in cell membrane, thereby this changes leads to increased expression of GGT which works to protect the cells against oxidative stress by increasing the intracellular glutathione (GSH) because that the GSH is work as a co-enzyme for many enzymes which responsible for protect the cells from damage caused by oxidation compounds and support their growth and survival<sup>(40)</sup>.

It appeared clearly through the present study that males were more susceptible to leukemia than females and that agrees with numerous studies by Khaled, (2013)<sup>(41)</sup> and AL-leban, *et al* (2015)<sup>(42)</sup>. The reasons than may be assist the male to acquire leukemia could be due to presence of the sex responsive gene near gene BCR-ABL which is located on a Philadelphia chromosome, that relatively protect the female against leukemia. This suggestion was supported by the study conducted by Alaa, *et al* (2009)<sup>(43)</sup> in Baghdad Teaching Hospital their studies demonstrated male dominance over female also might due to nature of their jobs and hormones.

## Conclusion

The present study concluded that the concentration of vitamin C and vitamin E in Iraqi patients with leukemia were decreased when compared to the normal controls, also there was decrease in Hb concentration and PCV level in patients when compared with healthy subjects, while the statistical analysis showed increase each of GGT and WBC count in patients, and a significant correlation was found between GGT levels and WBC count.

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## تقييم مستويات فيتامين C و E والمالون ثنائي الديهايد وبعض مكونات الدم وانزيم غاماغلوتاميل ترانسفيراز في المرضى المصابين باللوكيميا

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### الخلاصة:

اللوكيميا احد اكثر أنواع السرطان انتشارا في جميع انحاء العالم وفي كلا الجنسين وجميع الاعراق وبغض النظر عن المستوى المعاشي. إذ يؤثر على الانسجة المكونة للدم (نقي العظام). ولأهمية وخطورة المرض أجريت الدراسة لتقييم حالة الاجهاد التأكسدي. الكشف عن تأثير اللوكيميا على بعض متغيرات الدم. تقدير انزيم غاما غلوتاميل ترانسفيراز.

أجريت الدراسة في الفترة بين آب 2016 حتى شباط 2017 في مركز أمراض الدم / مستشفى اليرموك التعليمي . تم جمع عينات الدم من 70 مريضا عراقيا مصابا باللوكيميا و 37 شخصا طبيعيا كمجموعة سيطرة , تتراوح اعمارهم ما بين (6-68).

أظهر التحليل الاحصائي لثنائي الملون الديهايد زيادة كبيرة ( $P \leq 0.05$ ) في المرضى الذين يعانون من سرطان الدم مقارنة مع المجموعة الصحية. في حين كان هنالك انخفاض معنوي ( $P \leq 0.05$ ) في تركيز فيتامين C وفيتامين E في المرضى ( $0.49 \pm 7.10$ ;  $0.65 \pm 8.75$   $\mu\text{mol/L}$  على التوالي) بالمقارنة مع المجموعة الصحية ( $0.67 \pm 18.83$ ;  $0.88 \pm 25.83$   $\mu\text{mol/L}$ ). اظهرت نتائج انزيم غاما غلوتاميل ترانسفيراز ارتفاعا معنويا ( $P \leq 0.05$ ) في مرضى اللوكيميا عند مقارنتها مع المجموعة الصحية, أظهرت نتائج المتغيرات الدمية انخفاضا معنويا ( $P \leq 0.05$ ) في كل من حجم خلايا الدم المرصوفة في المرضى الذين يعانون من اللوكيميا مقارنة مع المجموعة صحية , وتركيز الهيموغلوبين في المرضى مقارنة مع المجموعة صحية. في حين كان هناك زيادة كبيرة ( $P \leq 0.05$ ) في إجمالي عدد الكريات البيض في مرضى اللوكيميا بالمقارنة مع المجموعة الصحية. وأظهرت نتائج الدراسة وجود علاقة إيجابية بين انزيم غاما كلوتاميل ترانسفيراز وخلايا الدم البيض، ولم نجد في المراجع المتاحة أي دراسة تشير إلى هذه العلاقة.

الكلمات المفتاحية: اللوكيميا, فيتامين C, فيتامين E, المالون ثنائي الديهايد, غاما غلوتاميل ترانسفيراز.