Correlation between malondialdehyde and metanephrine in patients with acute lymphoblastic leukemia

*Jwan A. Zainulabdeen**

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Abstract:

Acute lymphoblastic leukemia (ALL) is one of the most common diseases , so in this study the serum level of malondialdehyde and its relationship with metanephrine was investigated in acute lymphoblastic leukemia patients over one month of treatment.
Some biochemical parameters (serum glucose , total serum protein , malondialdehyde ,vitamin C, and metanephrine) changed as well as white blood cell count and blood hemoglobinlevelswere analyzed in sixty patients diagnosed with acute lymphoblastic leukemia over one month of treatment compared to healthy control group.
Statistically significant increases (p<0.01) in white blood cell (WBC) count, mean concentrations of malondialdehyde (MDA) (p< 0.05) and metanephrine (p< 0.001) were observed in contrast significant decreases in blood hemoglobin (p<0.001) ,random blood glucose (p<0.01) and total serum protein (p<0.01) were determined in ALL patients group than that control group. Meanwhile the results showed a positive correlation (p<0.001)between metanephrine and MDA in ALL patients in comparison to control. The results in the present study indicate a possible link between increased metanephrine levels of cells alterations due to oxidative damage.

Key words: Acute Lymphoblastic Leukemia, Metanephrine, Total serum Protein ,Malondialdehyde, and Vitamin C.

Introduction:

Acute lymphoblastic leukemia (**ALL**) is cancer of the white blood cells categorized by excess lymphoblasts [1].Acute lymphoblastic leukemia is most common in childhood with a peak occurrence at 2–5 years of age, and another peak in old age. The overall cure rate in children is about 80%, and about 45%-60% of adults have long-term disease-free survival. Both genetic and environmental risk factors have been implicated in the pathogenesis of this cancer of the hematopoietic system, but their implications have not been fully understood and appreciated [2]. Some of the environmental features that have been examined so far consist of ionizing radiation,infections, electromagnetic fields, chemicals, but only ionizing radiation has been consistently associated with childhood leukemia[3].Several study showed no association of family history of cancer with childhood ALL, while providing additional signal for an inverse association with family history of allergic disease[4].

Diet has been linked with many types of cancer, foods with high fat content may confer to the risk of childhood leukemia, whereas milk and dairy products might have a protective role [5]. A large percentage of children undergoing treatment for ALL have insufficient intakes of antioxidants and vitamin A. Lower intakes of antioxidants are associatedwith increases in the opposing side effects of chemotherapy. Greater vitamin C intakes were associated with fewer therapy delays, less toxicity, and fewer

*Department of Chemistry, College of Science, University of Baghdad, Iraq.

days spent in the hospital [6] ,meanwhile treatment strategies should be tailored by age[7].Metanephrine (MN) is metabolite of the adrenomedullary hormones epinephrine produced by action of catechol-O-methyl transferase (EC 2.1.1.6) on it,Vanillylmandelic acid synthesis from oxidative deamination of *O*-methylated catecholamine metabolites metanephrines, this occurs mainly in the liver and leaving very little else to escape into the hepatic venous effluent [6], MN is present in urine mainly as sulfate- and glucuronide- conjugated metabolites produced from free MN by the actions of conjugating enzymes. The measurement of plasma free metanephrines is considered to be the best instrument in the diagnosis of pheochromocytoma [6-9].

Reactive oxygen species (ROS) not only as beneficial substances such as in chemotherapy and cancer apoptosis [10,11], but have also established their role in carcinogenesis [10,12].Increased ROS formation and decreased competence of the antioxidant defense not only causes the permanent variation of bimolecular structures but also their functions[11,13].The autocatalytic process of oxidative destruction to polyunsaturated fatty acids caused by hydroxyl radicals and oxygen generates markers of lipid damage such as 4- hydroxynonenal and malondialdehyde (MDA) [14] .

The aim of this study was to evaluate the correlation between MDA (as the indices of lipid peroxidation) and MN in plasma of ALL patients.AS our knowledge this is the first study deals with MN in ALL disease.

Materials and Methods:

The patients of Acute Lymphoblastic Leukemia(ALL) as they were submitted to the Protection of Children Hospital Medical City in Baghdad, Iraq were selected for this study. The diagnosis for ALL based on the following findings: leukocyte count, age, involvement of tissues other than bone marrow. Different individuals were selected as control healthy groups. Venous blood samples (10) ml was drawn from (60) patients of ALL ranging between (1-16) years old, after one month postchemotherapy treatment.

Venous blood samples have been collected into three vacationer tubes, one containing EDTA for measurement of blood hemoglobin (Hb), WBC count. The plasma was separated by centrifugation at 3000 rpm for 15 minutes, then transferred immediately to a clean dry plain tube and anticoagulant tube for measuring metanephrine. The blood in the third part was allowed to clot for at least 10-15 min. at room temperature, centrifuged for (10) min. at (4000xg). Serum was removed for the measurement of biochemical parameters. Blood was obtained from (30) healthy individuals ranging in age between (1–16) years as a control group. They presented no acute or chronic diseases such as diabetes, or any immune dysfunction. Also, the control used in this study had normal leukocytes and other blood cell counts and made no use of pharmacological therapy.

The serum glucose and total serum protein levels were measured by spectrophotometric methods supplied by randox Diagnostic. Serum malondialdehydewas determined according to the modified method of Benge J.A.1978[15].Vitamin C levels were estimated by the method of Tietz [16]. The plasma metanephrinewere measured by Enzyme Linked Immunosorbent Assay (ELISA) (Demeditec Diagnostics GmbH, Germany).Protein electrophoresis was

carried using cellulose acetate paper. All statistical analyses in studies 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 $p< 0.05$ = significant, $p> 0.05$ = non-significant .Correlation analysis was used to test the linear relationship between parameters. ANOVA test was used to show the differences between variables of differentiated groups.

Results and Discussion:

The results presented in tables (1 and 2) are age and gender distribution of the study groups, respectively, based on the analysis of data on a total of 90 postchemotherapy cases which comprises the following: sixty cases diagnosis of ALL ,30male and 30 female , with age ranged from (1-16) years in comparison with healthy control: consisted of 30 cases healthy that were not complained from any disease.

Table 1: The age distribution of study groups

Age [year]	Patients group		Control group	
	No	%	No	%
1-8	33	55	16	53.33
9-16	27	44	14	46.67

*Significant at 0.05 level of significance.

Table 2: The gender distribution of study groups

	Patients group		Control group	
	No	%	No	%
Male	30	50	14	46.67
Female	30	50	16	53.33

*Significant at 0.05 level of significance.

The demographic and hematological changes that were observed among the age groups are presented in table (3).These results indicated that there was a significant increase in white blood cell (WBC) count ($p<0.01$) with a significant decrease in both hemoglobin (Hb) concentration ($p<0.001$) and PCV percentage ($p<0.001$)in sera of ALL patients group as compared with that of the control group, while a non-significant differences was observed according to the age.

Table 3: Demographic and hematological data of different studied groups (Mean±SD)

Characteristic	Patients group [n=60]	Control group [n=30]	*p* Value
Age [year]	7.53±3.48	7.00±4.09	>0.05
Hb[g/dl]	9.31 ± 1.4	11.57 ± 0.75	<0.001
PCV %	30.06±5.03	37.27±2.41	<0.001
WBC*10^3cell/ml	9.15±0.39	7.21±0.97	<0.01

*Significant at 0.05 level of significance.

Pathogenic mechanisms of anemia in cancer patients is hemolysis in erythrocytes, so the results in this study indicated that the Hb levels decreased significantly in the patients of and ALL after chemotherapy as compared to the healthy ($p<0.001$), table 3 , while the WBC counts were found to be increased significantly ($p<0.01$),butin 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.

Table (4) illustrated the biochemical parameters represented by [random serum glucose(R.S.G.),total serum protein (T.S.P.),MDA, vitamin C ,and MN levels of two studied groups ALL patients in comparison to control group].

Table 4: The biochemical parameters of different studied groups (Mean± SD)

Characteristic	Patients group [n=60]	Control group [n=30]	*p* Value
R.S.G[mg/dl]	79.92±16.43	91.23±11.51	<0.01
T.S. P[g/dl]	6.25 ± 1.10	6.95 ± 0.57	<0.01
MDA [nmol/ml]	2.46±0.24	1.55±0.21	<0.05
Vitamin C [mg/dl]	2.06±0.13	2.08±0.20	>0.05
MN[pg/dl]	84.69 ±29.75	48.75±7.77	<0.001

As shown from table (4) changes of R.S.G, T.S.P ,MDA, and MN levels were assessed by comparing control groups blood values with values obtained fromALL patient's ,where R.S.G, T.S.P decreased significantly (p<0.01,from 91.23 ±11.51 mg/dl to79.92±16.43mg/dl, and p<0.01 , from 6.95 ± 0.57g/dlto6.25 ±1.10g/dl, respectively), while MDA and MN concentrations increased significantly (p<0.05, from 1.55±0.21 nmol/ml to 2.46±0.24nmol/ml) , and(p<0.001,from 48.75±7.77pg/dl to 84.69 ±29.75pg/dl), respectively, while in contract there was no significant change in vitamin C levels between the two groups.

Also , the patients group had an approximate similar vitamin C concentration that of control (2.06±0.13 *vs.* 2.08±0.20 , p>0.05), that may associated with patient nutrition i.e. inadequate intakes of vitamin C because the requirement for this important antioxidant vitamin may be greater in children with ALL [6]especially during chemotherapy.

The patients in the present study reflected random hypoglycemia (79.92±16.43 *vs.* 91.23±11.51 , p<0.001) during induction therapy, this is in agreement with previous study in which A patient with acute lymphoblastic leukemia repeatedly developed hypoglycemia during chemotherapy. Comparison of serum glucose trends between chemotherapy with and without L-asparaginase demonstrated a strong association between L-Asp and hypoglycemia. Critical blood sampling during hypoglycemia indicated hyperinsulinism, suggesting that L-Asp induced hypoglycemia in the patient through inappropriate insulin secretion [17], also tumor cells overexpress hexokinase or insulin like growth factor-1 increase glycolysis, which raises the glucose level, and consequently, proliferates tumor cells again. Since tumor cells use anaerobic metabolism despite the existence of oxygen, a lot of glucose turns to lactic acid [18-20].

Oxidative stress studies on various types of cancer have indicated increased MDA levels as compared to normal individuals [11, 21-23]. These findings correlate with the levels of MDA measured in our study(28.10 ± 2.90 nmol /L vs. 9.20 ± 0.30 nmol/L) where a significant increase (p< 0.0001) was observed in ALL patients as compared to healthy individuals ,table 4 ,these results were in accordance with those of earlier studies [11,24,25].

In order to clarify the correlation between these two parameters (MN and MDA) within each of the studied groups, the results were reanalyzed by using linear regression analysis. A positive correlation was established between plasma MN and MDA in sera of patients group under studied only (n=60,r=0.67, p<0.01), but not for control group(n=30,r=0.08, p>0.05) ,(figure 1).

A direct correlation between MDA and cell proliferation with increased lipid damage in highly proliferated cells has been noted [26]. On another study showed that the elevated DNA, lipid and protein oxidation may have occurred as a

result of a weakened defenses system [27].This suggestion supports the present findings where a decrease in T.S.P(6.25 ± 1.10 *vs.* 6.95 ± 0.57,p<0.01) observed may be due to the increased lipid peroxidation observed. However, it should be noted that .It has been reported that certain lipid peroxidation products like MDA can attack the sulfhydryl groups and the amino group in proteins [28].

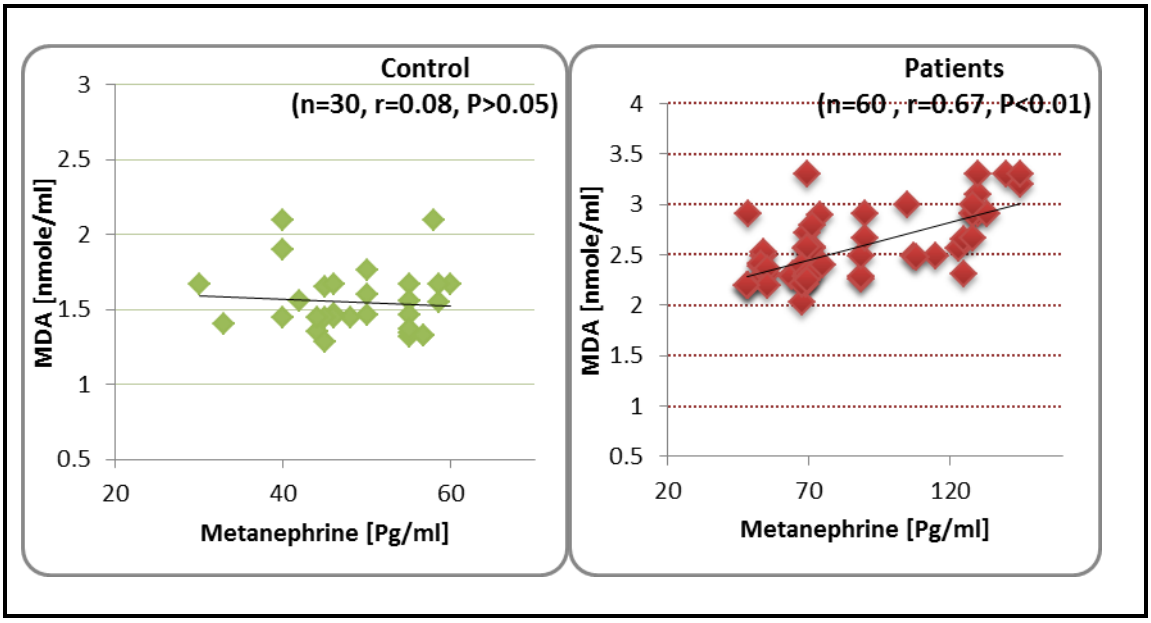

Fig. 1: The correlation between plasma MN and MDA insamples of patients group and control group.

The present study showed that an increase in the mean level of MN (28.10 ± 2.90 nmol/L, p< 0.0001) in the ALL patient group when compared to control group as shown in table 4, that leads to a significant correlation between MN (n=60 , r=0.67,p<0.001) in these samples, however, the correlation was not significant for control group (n=30, r=0.08,p>0.05) , (figure 1) . This positive correlation confirmed that the oxidative stress presented in treated patients with ALL disease demonstrated by the increased levels of MDA as a consequence of abnormality in antioxidative metabolism due to the cancer process which significantly progresses by the effect of the chemotherapymight lead to compensatory increased level of the MN in these patients.

Results of the biochemical parameters for the two studied groups according to age, and gender are given on tables (5 and 6), respectively .It is clear from the results in table (5)that there were no significant differences (p>0.05) of all biochemical parameters (except for MN levels ,p<0.05) in samples of ALL patient group in comparison to that of the control group , while the results presented in table (6) reveals non- significant difference (p>0.05) in all biochemical parameters in samples of the two studied groups.

Table 5:The biochemical parameters in patients group according age (Mean± SD)

Characteristic	Patients group [Age 1-8 year]	Control group [Age 9-16 year]	*p* Value
R.S.G[mg/dl]	77.15±13.5	83.30±19.13	>0.05
T.S. P [g/dl]	6.13 ± 1.10	6.39 ± 1.13	>0.05
MDA [nmol/ml]	2.43±0.19	2.49±0.30	>0.05
Vitamin C [mg/dl]	2.08±0.13	2.04±0.12	>0.05
MN[pg/dl]	80.44 ±26.69	89.88±32.87	<0.05

Table 6:The biochemical parameters in patients group according gender (Mean± SD)

Characteristic	Patients group [female]	Control group [male]	*p* Value
R.S.G[mg/dl]	78.50 ±12.32	81.33±19.83	>0.05
T.S. P [g/dl]	6.20 ± 1.34	6.30 ± 0.82	>0.05
MDA [nmol/ml]	2.40±0.22	2.52±0.25	>0.05
Vitamin C [mg/dl]	2.07±0.11	2.05±0.14	>0.05
MN [pg/dl]	84.51 ±30.01	84.87±29.99	>0.05

To differentiate between protein patterns cellulose acetate electrophoresis was carried out on sera samples of control and patients groups then separated serum protein bands were detected using ponceau S-stain., figures 2.This figure indicated that the sera was separated into distinct bands by which the separation of different proteins is based on the differences of both molecular size and the charge of these proteins and there is a clear difference in proteins band intensity, which reflects the significant variation in proteins concentration between the studied groups.

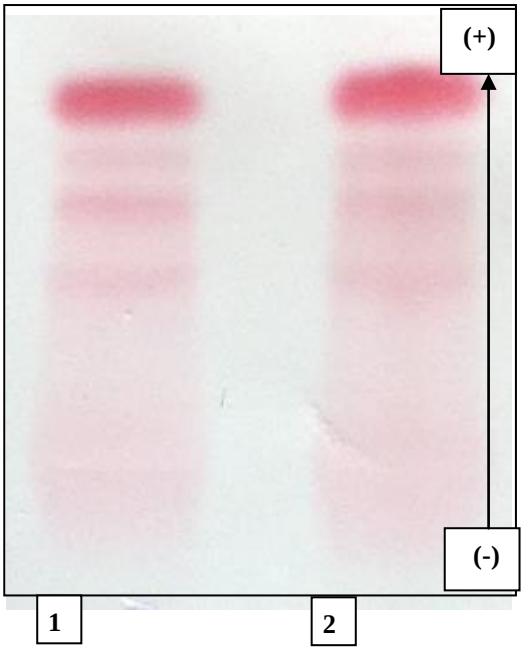

Fig.2. Electrogramof proteins profile samples , (The crude samples that applied were:1: pooled crude sera (patient) ;2: pooled crude sera (control).

The present results suggest an oxidative stress presented in patients with ALL demonstrated by the increased levels of MDA as a consequence of abnormality in anti-oxidative metabolism due to the cancer process which may be increased after chemotherapy. The oxidative stress might lead to compensatory increased level of the MN in these patients Moreover, to maintain MN levels constant, the rate of MN turnover(i.e., loss of MN due to metabolism or escape to the bloodstream) must be balanced by an equal rate of synthesis, also the increasing metanephrin's levels may be due to the decreases in catabolism in compression with anabolism .Also the concentration of epinephrine increase the degradation of glycogen [29], so the decreases in R.B.S. levels may stimulate the excretion of epinephrine, as a consequence increasing MN levels ,table 4.

Comparisons of control to ALL patient's plasma concentrations of

R.S.G. ,T.S.P. , MDA, and vitamin C levels show negligible or relatively small differences according to age or gender (tables 5and 6), while MN levels differ significantly according to age.

The results of the present study seem that chemotherapy causes these changes as a result of huge production of ROS in the patients with ALL.

Assessment the effect of antioxidant supplementation to improve antioxidant status in these patients is worth to be carried out in future. Moreover to evaluate the diagnostic performances of plasma total metanephrine in ALL disease, it's levels must be estimated before chemotherapy and in combination with24-h urinary metanephrine.

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العلاقة بين المالونديالدهيدوالميتانيفرين في المرضى الذين يعانون من سرطان الدم الليمفاوي الحاد

جوان عبد المحسن زين العابدين*

*قسم الكيمياء ،كلية العلوم، جامعة بغداد

الخلاصة :

يعد مرض سرطان الدم الليمفاوي الحاد من الامراض الشائعة ولذلك تهدف الدراسة الحالية لتحديد مستوى المصل من المالونديالدهيد وعلاقته مع ميتانيفرين في مرضى سرطان الدم الليمفاوي الحادبعد شهر واحد من العلاج.

تضمنت الدراسة الحالية قياس بعض العوامل البيوكيميائية (الكلوكوز في مصل الدم ، البروتين الكلي في مصل الدم ، المالونديالدهيد ، فيتامين C ، والميتانيفرين) وكذلك عدد خلايا الدم البيضاء و الهيموكلوبين في أمصال 60مريضا مصابة بسرطان الدم الليمفاوي الحادخلال شهر واحد من العلاج ومقارنتها بمجموعة الضبط.

أظهرت الدراسة زيادة معنوية في عد خلايا الدم البيضاء والمالونديالدهيد والميتانيفرين وفي المقابل لوحظ انخفاض كبير في مستوى الهيموغلوبين بالاضافة الى مستويات السكرالعشوائي و البروتين الكلي في مصل الدم،كما لوحظ وجودعلاقة ترابطية معنوية بين مستويات المالونديالدهيد مع الميتانيفرين في المرضى المصابين بسرطان الدم الليمفاوي في حين لم يكن هناكعلاقة ترابطية معنويةلمجموعة الضبط.

تشير النتائج التي تم التوصل اليها الى وجود صلة واضحة بين زيادة مستويات الميتانيفرين مع تاثر الخلايا بالضرر التأكسدي.