

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

Seenaa A. al- Hammami*

Received 2, June, 2014

Accepted 23, June, 2014



This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License](https://creativecommons.org/licenses/by-nc-nd/4.0/)

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 amino transferase(AST), Alanin amino transferase (ALT), and Malondialdehyde (MDA) in blood which can be concenter 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

*Department of Chemistry, College of Science for Women, University of Baghdad, Iraq

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 cases 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 compounds 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, septicaemias, 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 concenter 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 $P < 0.05$ = significant, $P > 0.05$ = 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.

Characteristic	Patients group mean \pm SD N=50	Control group mean \pm SD N=30	p Values
Age[year]	15.47 \pm 3.96	15.3 \pm 4.97	N.S
Hb [g/dl]	9.11 \pm 1.42	11.62 \pm 0.62	<0.001
PCV %	29.42 \pm 4.99	38.33 \pm 1.91	<0.001
Total protein [g/dl]	5.88 \pm 1.12	7.03 \pm 0.62	<0.001
Glucose [mg/dl]	79.28 \pm 18.98	80.06 \pm 11.28	N.S
Urea [mg/dl]	30 \pm 5.30	29.43 \pm 5.84	N.S
Creatinine [mg/dl]	1.01 \pm 0.18	0.92 \pm 0.19	N.S
ALT [IU/l]	46.58 \pm 1.15	13.56 \pm 3.13	<0.001
AST [IU/l]	13.76 \pm 1.76	13.2 \pm 1.88	N.S
MDA[μ mole/l]	2.59 \pm 0.26	1.52 \pm 0.23	<0.01
Vit. C [mg/dl]	2.07 \pm 0.16	2.11 \pm 0.18	N.S

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 lipids 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 observed 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's 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.

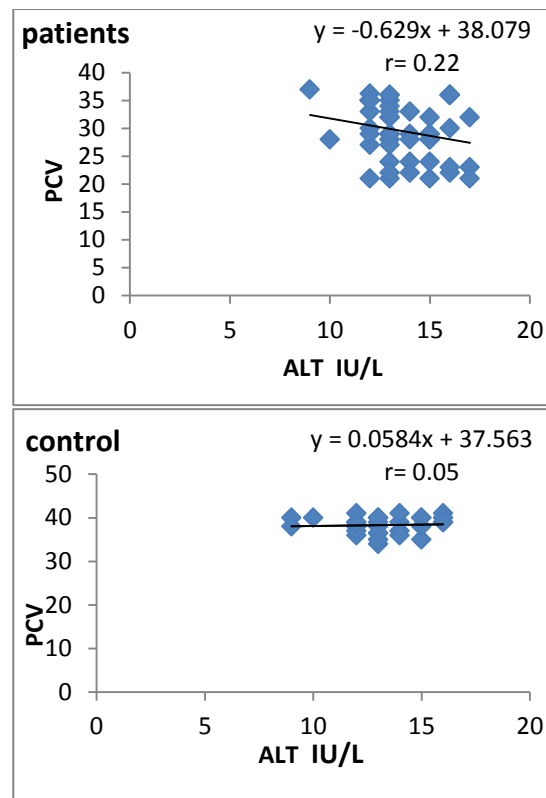


Fig. 2: Correlation between PCV with ALT in Leukemia patients and Control.

References:

- 1-Hoffbrand A. V., Moss P. A. H. and Pettit J. E. 2006. Essential Haematology, 5th Ed, 157- 159:167.
- 2-Ravindranath Y. 2003. Recent advances in pediatric acute lymphoblastic leukemia. Curr. Opin. Oncol :15: 23-35
- 3-Pui C.H.; Evans W. E. 2006. Acute lymphoblastic leukemia. N Engl J Med354:166-78.
- 4-Conter V; Arico M; Valsecchi MG; Conter V; Arico M; Valsecchi MG; etal. 2000. Leukemia, Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) acute lymphoblastic leukemia studies 14:2196-204
- 5-Elizabeth Raetz, 2014. Acute Lymphoblastic Leukemia, University of Utah ,Huntsman Cancer Institute, Primary Children's Hospital, Salt Lake City, UT 2014.2

- 6- Conter V; Rizzari C; Sala A; Chiesa R; Citterio M and Biondi A, 2014. Acute Lymphoblastic Leukemia. Orphanet encyclopedia. 2004 December.
- 7- Pui C H; Robison L L; 2008. Acute lymphoblastic leukemia. *Lancet.*; 371(9617):1030-43
- 8- Warburg O. 1956. On the origin of cancer cells. *Science* ; 123: 309–314.
- 9- John A P. 2001. Dysfunctional mitochondria, not oxygen insufficiency, cause cancer cells to produce inordinate amounts of lactic acid: the impact of this on the treatment of cancer. *Med Hypotheses* ; 57: 429–431.
- 10- Silverman L B; and Sallan S E. 2003. Newly diagnosed childhood acute lymphoblastic leukemia. *Curr Opin Hematol.*;10:290-296
- 11- Dworzak M N, and Panzer-Grumayer E R. 2003. Flow cytometric detection of minimal residual disease in acute lymphoblastic leukemia. *Leuk Lymphoma.*;44:1445-1455.
- 12- Ritu A.; William H. ; and Poul B . 2013. Effect of hematocrit on analytical quantification using dried blood spot technology for pharmaceutical bioanalysis, Aligent technologies , Inc,2013
- 13- Segal I; Rassekh S R; Bond M C; Senger C; and Schreiber R A. 2010. Abnormal liver transaminases and conjugated hyperbilirubinemia at presentation of acute lymphoblastic leukemia. *Pediatr Blood Cancer* ;55(3):434-9
- 14- Santana VM; Dodge RK; Crist W M; Rivera GK; Behm FG; et al. 1990. Presenting features and treatment outcome of adolescents with acute lymphoblastic leukemia. *Leukemia* 4:87–90
- 15- Reiter A; Schrappe M; Ludwig W D; Hiddemann W; Sauter S; and Henze G; et.al. 1994 .Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. *Blood* 84:3122–33
- 16- Donadieu J; Auclerc MF; Baruchel A; Leblanc T, Landman -Parker J, and Perel Y; et al. 1998. Critical study of prognostic factors in childhood acute lymphoblastic leukaemia: differences in outcome are poorly explained by the most significant prognostic variables. Fralle group. French Acute Lymphoblastic Leukaemia study group. *Br J Haematol* 102:729–39.
- 17- Hann I; Vora A; Harrison G; Harrison C; Eden O; and Hill F; et al. 2001. Determinants of outcome after intensified therapy of childhood lymphoblastic leukaemia: results from Medical Research Council United Kingdom acute lymphoblastic leukaemia XI protocol. UK Medical Research Council's Working Party on Childhood Leukaemia. *Br J Haematol* 113:103–14.
- 18- Ullagaddi R.; Iyer S.; Rao Ra.; and Bondada A., 2013. Oxidative Stress and Antioxidant Status in Acute and Chronic Myeloid Leukemia Patients, *OJOB* , 3, 17-22.
- 19- Willcox J K; Ash SL; Catignani GL, 2004. Antioxidants and prevention of chronic disease, *Crit Rev Food Sci Nutr.* ;44(4):275-95
- 20- Nishikimi M; Fukuyama R; Minoshima S; Shimizu N; and Yagi. K.; 1994. Cloning and chromosomal mapping of the human nonfunctional gene for L-gulonogamma-lactone oxidase, the enzyme for Lascorbic acid biosynthesis missing in man. *J Biol Chem*; 269: 13685-13688.
- 21- Chan A. 1993. Partners in defense: vitamin E and vitamin

- C.Can J Physiol Pharmacol; 71: 725-731.
- 22- Levine M; Rumsey SC; Wang Y; Park JB; Daruwala R. and Vitamin C. In Stipanuk MH (ed): 2000 "Biochemical and Physiological Aspects of Human Nutrition." Philadelphia: W B Saunders, pp 541-567
- 23- Buettner G. R. and Moseley P.L. 1993. EPR spin trapping of free radicals produced by bleomycin and ascorbate. Free Radic Res Commun; 19: S89-S93
- 24- Champlin R. and Gale R P. 1989. Acute lymphoblastic leukemia: Recent advances in biology and therapy, Blood 73: 2051-2066.
- 25- Nishikimi M. and Yagi K. 1996. Biochemistry and molecular biology of ascorbic acid biosynthesis. Subcell Biochem; 25: 17-39.
- 26- Esterbauer H; Schaur R. J, and Zollner H, 11 1991. Chemistry and biochemistry of 4-hydroxynoneal malondialdehyde and related aldehydes, Free Radicals Biol. Med. 81-128.
- 27- Bielski B H, Richter H W, and Chan PC. 1975. Some properties of the ascorbate free radical. Ann N Y Acad Sci; 258: 231-237
- 28- Satoh K 1978. Plasma lipid peroxide in cerebrovascular disorders determined by new colorimetric method. Clin Chim Acta, 90, 37-43.
- 29- Sharma Poudel; Karaki L. 2007. Abnormal hepatic function and splenomegaly on the newly diagnosed acute leukemia patients. J Nepal Med Assoc; 46(168):165-9
- 30- El-Sabagh M. E; Ramadan K. S; El-slam I. M. A, and Ibrahim A. M. 2011. Antioxidants Status in Acute Lymphoblastic Leukemic Patients, American Journal of Medicine and Medical Sciences. 6-1,(1).1
- 31- Veena S. ; Munish K.; Kiran D.; Rakesh D.; and Ragini S. 2012. Erythrocytic Pyruvate Kinase and Malondialdehyde Levels in Acute Leukaemia Patients. JCDR .6:3-(361-363)
- 32- Sheikh N.; Abid R.; Qureshi AW. and Basheer T. 2012. Expression of Low Molecular Weight Proteins in Patients with Leukaemia, West Indian Med J 61 (3): 235-239
- 33- Halton JM.; Atkinson SA. and Barr RD. 1998. growth and body composition in response to chemotherapy in children with acute lymphoblastic leukemia, Int J Cancer Suppl 11, 81-84.
- 34- Khan AU.; Sheikh MU. and Intekhab K. 2006. Effect of hypoproteinemia on treatment outcome in children with acute lymphoblastic leukemia, J Ayub Med Coll Abbottabad 18(2):53-56.

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

سيناء عبود الحمّامي

قسم الكيمياء - كلية العلوم للبنات - جامعة بغداد

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. ويمتاز بوجود عدد كبير من خلايا الدم البيضاء غير الناضجة في دم الطفل المصاب ونخاعه العظمي . كما ويصيب هذا المرض البالغين أيضاً إلا ان علاج الاطفال يكون مختلفاً. حيث يظهر على الاطفال اعراضاً متسببةً عن تسلل خلايا الدم البيضاء غير المتخصصة في نخاع العظم الى الجهاز اللمفاوي والمواقع خارج نخاع العظم كالجهاز العصبي المركزي. من أكثر اعراض ALL شيوعاً الاعياء (50%)، الشحوب (25%)، الحمى (60%) وخسارة الوزن (26%)، كما أن تسلل كريات الدم البيضاء غير المتخصصة الى تجاويف نخاع العظم وغشاءه (السحاق) غالباً ما يؤدي الى آلام العظام (25%)، كما يسبب اضطراباً في تكوّن خلايا الدم الطبيعية ، ويلاحظ أن 75% من المرضى يصابون بنقص في الصفائح الدموية بتعداد أقل من 100,000 ، وانخفاض مستوى Hb لدى 40% من الأطفال المصابين الى أقل من 7 mg\dl، كما لوحظ لدى 20% من الحالات المرضية ارتفاع تعداد كريات الدم البيضاء الى أكثر من 500,000\mm³. كما وجد أن نقص كريات الدم البيضاء المتعادلة (أقل من 500) شائعاً ومصحوباً بزيادة خطر الإصابة بالعدوى. هدف هذه الدراسة هو التحقق من بعض المؤشرات في الدم والتي يمكن اعتبارها دلالة للإصابة بسرطان الدم اللمفاوي الحاد. في هذا البحث تم دراسة مستويات PCV، Hb، البروتين الكلي في مصل الدم، سكر الدم ، اليوريا، الكرياتينين، انزيمي AST و ALT و بعض مضادات الأكسدة. جمعت النماذج من 50 طفل مريض بسرطان الدم اللمفاوي الحاد والذين تتراوح أعمارهم بين (1-16) سنة بعد شهر من تعاطيهم للعلاج التحفيزي بالمقارنة مع 30 عينة اخذت من اشخاص اصحاء بنفس العمر. وجدت فروقات ملحوظة في انزيم ALT ، البروتين الكلي في مصل الدم، MDA، Hb، PCV عند المقارنة مع مجموعة السيطرة. كما لوحظ وجود علاقة سلبية ما بين AST و PCV لدى مجموعة المرضى (r = 0.22 , p<0.05)، بينما لم يتم رصد علاقة ملحوظة في مجموعة الأصحاء.

الكلمات المفتاحية: سرطان الدم اللمفاوي الحاد، انزيم AST، MDA.