Evaluation of Some Biochemical Parameters and Hormones In Patients with Acute Myeloid Leukemia in Iraq

Hind Mahmood Jumaah*[1], Jabbar H. Yenzeel[1] and Mohammad G. Mehdi[2]

[1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

[2]College of Medicine, Baghdad University, Baghdad, Iraq

Abstract

The effect of myeloid leukemia, especially cute myeloid leukemia (AML), has been widely noticed on the parameters of liver and kidney functions and the levels of certain hormones. This study aimed to evaluate a number of biochemical parameters of liver and kidney functions and hormones in Iraqi subjects with newly diagnosed acute myeloid leukemia. Eighty newly diagnosed AML adult patients (40 males and 40 females) and forty healthy individuals (20 males and 20 females) with an age range of 16-75 years were involved in this study during their attendance at the Hematology Department of Baghdad Teaching Hospital/ Medical city in Baghdad province from March 2019 to February 2020. Blood samples were collected from all subjects for the determination of serum levels of the parameters of liver function parameters., kidney function , lactate dehydrogenase (LDH), and Erythropoietin (EPO). The results showed that the serum levels of liver function parameters (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) had highly significant increases (p< 0.01) in AML patients (85.87±2.49 , 53.93±1.76, 150.87±7.04 U/L, respectively) as compared to the control (30.58 ±2.04, 22.89 ±0.97, 75.51 ±2.12 U/L, respectively). Also, the level of kidney function parameters (blood urea, creatinine and uric acid) showed highly significant increases (p< 0.01) in AML patients (58.82 ±1.49, 1.831 ±0.05, 8.34 ±0.15 mg/dl, respectively) as compared to the control (31.10 ±1.03, 0.850 ±0.02, 4.81 ±0.14 mg/dl, respectively). In addition, the level of LDH showed a highly significant increase (p< 0.01) in the patients with AML (657.72 ±80.76 U/L) as compared to the control (166.05 ±6.15 U/L). Moreover, the level of EPO showed a highly significant increase (p<0.01) in the patients with AML (11763.80 ±329.46 pg/ml) as compared to the control (316.94 ±34.42 pg/ml).

Key words: Liver and kidney function, AML, LDH, EPO.

<div dir="rtl">

تقييم بعض المعايير الكيموحيوية والهرمونات في مرضى سرطان الدم النخاعي الحاد في العراق

هند محمود جمعة*[1] , جبار حميد ينزيل[1] , محمد غانم مهدي[2]

[1]جامعة بغداد، كلية العلوم، قسم علوم الحياة, العراق، بغداد

[2]جامعة بغداد، كلية الطب، العراق، بغداد

الخلاصة

لوحظ تأثير ابيضاض الدم وخاصة النخاعيني في وظائف الكبد والكلى وبعض الهرمونات. هدفت الدراسة الى تقييم مستوى بعض المعايير الكيموحيويه لوظائف الكبد والكلى وبعض الهورمونات في مرضى سرطان الدم

</div>

*Email: hind.mahmood.jumash@gmail.com

النخاعي الحاد في العراق. ثمانون بالغاً (40ذكراً و40 أنثى)تم تشخيصهم حديثاً بمرض أبيضاض الدم النقوي الحاد، واربعون من الاصحاء 20(ذكراً و 20 أنثى) معدل اعمار المرضى ,والاصحاء تتراوح بين16– 75 عاما. تم شمولهم في هذه الدراسة خلال حضورهم الى قسم أمراض الدم في مستشفى بغداد التعليمي في مدينة الطب في محافظة بغداد للفترة من اذار 2019 الى شباط 2020. جمعت عينات الدم من المرضى الذين يعانون من أبيضاض الدم النقوي الحاد حديثاً والاصحاء لتقدير مستوى وظائف الكبد والكلى ونازعة هيدروجين اللاكتات، وهرمون الإريثروبويتين.

اظهرت النتائج حصول ارتفاع معنوي عالي (P<0.01) في مستوى وظائف الكبد (ALP ,AST , ALT) في مرضى(L/U 150.87±7.04; U/L 53.93±1.76; AML85.87±2.49U/L على التوالي) مقارنه مع مجموعة السيطرة (U/L 2.12± 75.51;U/L 0.97± 22.89;U/L 2.04± 30.58 على التوالي). أظهر مستوى معايير وظائف الكلى (يوريا الدم ,الكرياتينين وحمض البوليك) ارتفاعاً معنوياً عاليا (P<0.01) في مرضى AML (mg/dl 0.15± 8.34;mg/dl 0.05± 1.831;mg/dl 1.49± 58.82 على التوالي) مقارنه مع الاصحاء (mg/dl 0.14± 4.81;mg/dl 0.02± 0.850;mg/dl 1.03± 31.10على التوالي). بالإضافة الى ذلك , أظهر مستوى LDH زيادة معنوية عالية (P<0.01) في المرضى AML (657.72 U/L 80.76±مقارنة بجموعه السيطرة (U/L 6.15± 166.05).و أظهر مستوى EPO زيادة معنوية عالية (P<0.01) في المرضى (pg/ml 329.46 ±11763.80)AML مقارنة بمجموعة السيطرة (316.94 ±34.42 pg/ml).

Introduction

Leukaemia is a haematological malignancy that occurs when a certain aspect of the division or life span of a blood cell or its precursor is regulated incorrectly [1]. The cell uncontrollably begins to proliferate and forms a large cell population derived from a single cell [2]. Haematological malignancies are cancers that affect the blood, bone marrow, and lymph nodes. This classification includes various types of leukemia (acute lymphocytic (ALL), chronic lymphocytic (CLL), acute myeloid (AML), chronic myeloid (CML), myeloma, and lymphoma (Hodgkin's and non-Hodgkin's (NHL)) [3,4].

Leukaemia refers to unregulated proliferation without differentiation of the progenitor cells. A block of differentiation results in the accumulation of immature cells which fail to mature fully and then die. Proliferating immature cells, which are stopped in differentiation and escape immune surveillance, ultimately dominate the bone marrow and invade other tissues and organs, which can lead to death [5].

In AML, infiltration has been observed both in the tract of the portal vein and in the sinusoid of the liver; extremely large infiltration of leukemic liver cells can appear as a fulminant liver failure [6]. Aminotransferases are normally present in the serum in low concentration. These enzymes are released into the blood in greater amounts when there is damage to the liver cell membrane resulting in increased permeability [7, 8]. In the case of hepatic infiltration by leukemia cells, the activation of ALP and gamma-glutamyl transferase (GGT) is high [9].

Blood urea nitrogen (BUN) acts as an important sign for the condition of the disease. Compared to the control group, BUN levels were found to be elevated in leukemia patients [10]. Research studies have shown that not only AML and ALL but also other types of chronic leukemia, such as CLL and acute neutropenia, have been associated with the mortality of patients with leukemia [10,11]. The findings of a current study suggest that the measurement of BUN levels may be useful for predicting renal outcomes [12].

LDH is a cytoplasmic enzyme found in nearly all tissues, but at rising concentrations in muscles, liver, and kidneys. Moderate levels of this enzyme are also present in red blood cells [13]. The enzyme 's function is to catalyze the reversible conversion of lactate to pyruvate by reducing NAD to NADH and vice versa [14]. During cell damage, LDH is progressively elevated, due to a cellular disorder occurring in acute leukemia, along with overgrowth of normal malignant cells [15].

LDH is elevated in the cases of myocardial or pulmonary infarction, leukemia's, hemolytic anemia, non-viral hepatitis, sickle - cell anemia, lymphoma, renal infarction, acute pancreatitis, and any case that results in the leaking of cytoplasm [16, 17].

Erythropoietin is a glycoprotein hormone released primarily by interstitial fibroblasts in the kidney, in the rate of 90%, in close association with peritubular capillaries and epithelial tubules [18,19]. Also, a limited contribution of 10% is conferred by the liver and other tissues, such as lung, brain , heart, spleen and, retina, which yield only modest levels depending on physiological and pathological changes [20]. EPO is produced in response to tissue hypoxia or severe anemia, where its main function is to control the process of blood production in the bone marrow [21, 22]. The present study was undertaken to assess the serum levels of liver and kidney function parameters, LDH, and EPO in a sample of Iraqi AML patients.

Materials and Methods

This study was conducted at the Hematology Department of Baghdad Teaching Hospital in Baghdad Medical City during the period from March 2019 to February 2020. Eighty adult newly diagnosed AML patients, including 40 males and 40 females, and forty healthy participants, including 20 males and 20 females, with an age range of 16-75 years were enrolled in this study.

Four ml of blood were collected from the cubical vein using 5 ml disposable needles and syringes. The blood was dispensed in a gel tube and left to clot for 10 minutes at room temperature, after which the serum was separated by centrifugation at 3000 rpm for 10-15 minute and preserved in deep freezer at -20 C before analysis. The kits of ALT, AST, ALP, blood urea, uric acid, and LDH (Siemens, Germany) are based on automated bichromatic endpoint method , while creatinine kit (Linear, Spain) was prepared based on manual measurement. The ELISA kit (Mybiosource, USA) used for the estimation of EPO hormone is designed for an accurate quantitative measurement. Statistical package for social science (SPSS/PC version 18) software was used to analyze the obtained data. Differences among means were computed using the t- test with a p- value $\leq$ 0.05.

Results and Discussion

The serum levels of liver function parameters (ALT, AST, ALP) showed highly significant increases ($p \leq$ 0.01) in patients with AML (85.87$\pm$2.49, 53.93$\pm$1.76, and 150.87$\pm$7.04 U/L, respectively) as compared to the control (30.58 $\pm$2.04, 22.89 $\pm$0.97, and 75.51 $\pm$2.12U/L, respectively), as illustrated in table (1).

Table 1-Levels of liver function parameters (ALT, AST and ALP) in the sera of AML patients and control.

Group	Mean $\pm$ SE		
	ALT U/L	AST U/L	ALP U/L
Patients	85.87 $\pm$2.49	53.93 $\pm$1.76	150.87 $\pm$7.04
Control	30.58 $\pm$2.04	22.89 $\pm$0.97	75.51 $\pm$2.12
t-test	7.62 **	5.18 **	20.22 **
p-value	0.0001	0.0001	0.0001
** ($p \leq$0.01).			

The present study found that the patients with myeloid leukemia had increased ALT and AST levels, which is consistent with previous studies that showed significant increased serum levels of both enzymes in patients with leukemia, viral hepatitis, and liver cirrhosis [23]. In leukemia patients, the high activity of these enzymes may be due to hepatic infiltration. An infiltrative disorder that results from a defect in the membranes of mitochondria and cytoplasm is proportional with increased levels of AST [24]. Increased ALT and AST levels in leukemia patients are caused by hepatic injury [25]. In addition, the elevation in the number of leukemic cells leads to an increase in the concentration of transaminase enzymes [26].

Moreover, in this study, the increase in ALP activity was similar to the results of earlier studies [27, 28], which reported that the increase in ALP activity could be due to either direct infiltration of leukemia cells into the liver as a result of damages in the mitochondrial and cytoplasmic membranes or to liver tissue damage due to a certain immune response. However, our results are not consistent with the findings of Lee *et al*. [29], who reported that the damaged liver cells fail to produce ALP.

The serum levels of kidney function parameters (blood urea, creatinine, and uric acid) were significantly increased (p≤ 0.01) in AML patients (58.82 ±1.49, 1.831 ±0.05, 8.34 ±0.15 mg/dl, respectively) compared with the control (31.10 ±1.03, 0.850 ±0.02, 4.81 ±0.14 mg/dl, respectively) as illustrated in Table-2.

Table 2-Levels of kidney function parameters (blood urea, creatinine and uric acid) in the sera of AML patients and control.

Group	Mean ± SE		
	Blood urea mg/dl	Creatinine mg/dl	Uric acid mg/dl
Patients	58.82 ±1.49	1.831 ±0.05	8.34 ±0.15
Control	31.10 ±1.03	0.850 ±0.02	4.81 ±0.14
t-test	4.47 **	0.164 **	0.472
p-value	0.0001	0.0001	0.0001
** (p≤0.01).			

The mean levels of serum urea and uric acid showed highly significant (p≤ 0.01) increases in the patient with AML as compared to the control. Our results agree with those of a previous study [30], which showed that serum urea and uric acid concentrations in patients with AML before treatment were statistically significantly higher than those in the control group. This is because uric acid in the serum is the result of the breakdown of the nucleic acids of leukemia cell and may be a marker of disease aggression [31,32].

High serum uric acid levels, including those observed in gout and articular degenerative disorders, as well as blood vessel inflammation and atherosclerosis, have been shown to play an important role in several disease states [33]. In patients with leukemia, multiple complications may cause clinically advanced hyperuricemia, including tumor lysis syndrome, drug adverse reactions, and renal dysfunction. Further research indicates that the increase in serum uric acid was caused by under-excretion due to renal dysfunction in some patients [32].

The level of creatinine demonstrated a highly significant increase in patients with AML compared with the control. This result agrees with that reported by an earlier work [34], which showed that creatinine level was highly significantly increased (p<0.01) in AML patients. Furthermore, because creatinine is an endogenous molecule, its metabolism is subject to interpersonal variations depending on various factors [35]. Creatinine is still the widely agreed criterion for the diagnosis of renal failure, but we must bear in mind that its importance represents the functioning of the kidney and not necessarily the existence of actual injury [36].

Other finding in this study revealed that serum LDH level had a highly significant increase (p≤ 0.01) in the patients with AML (657.72 ±80.76 U/L) as compared to the control (166.05 ±6.15 U/L), as shown in Table-3.

Table 3-Levels of LDH and EPO in the sera of AML patients and control

Group	Mean ± SE	
	LDH U/L	EPO pg/ml
Patients	657.72 ±80.76	11763.80 ±329.46
Control	166.05 ±6.15	316.94 ±34.42
t-test	229.74 **	937.76 **
p-value	0.0001	0.0001
* (p≤0.05), ** (p≤0.01).		

This result agrees with those published by Elbossaty [37] and Merza *et al*. [38], who reported that the mean LDH level at presentation was extremely significantly increase as compared with the control. This may be due to a cancer burden activity that has reversed the function and turnover of leukemia cells. LDH level can be adopted as a useful tool , not only as a differentiation marker for different types of leukemia, but also for patient follow-up during the treatment time.

Increased cellular LDH activity represents a change towards anaerobic metabolism and increased glycolysis in the cytoplasm of malignant cells, followed by high cell turnover. However, lactate dehydrogenase is a key enzyme in the system of producing energy in tumor cells. It stimulates the conversion of pyruvate to lactate under hypoxic conditions [39]. Because of its role in anaerobic metabolism, tumor cells grow even after rapid proliferation, leading to low oxygen conditions in the microenvironment of the tumor [40].

The serum level of EPO, the hormone of the kidney, showed a highly significant increase ($p \leq 0.01$) in the patients with AML (11763.80 ±329.46 pg/ml) compared with the control (316.94 ±34.42 pg/ml), as shown in table (3). This result is in agreement with that of Molica *et al*. [41] in their study of patients with leukemia, who declared that the elevated level of EPO may be due to the disorder itself, which is characterized by an irregular proliferation of immature cells, called blasts, that may interfere with the development in the bone marrow of natural blood cells. Therefore, it contributes to erythrocytopenia, leukocytopenia, and thrombocytopenia. Also, many symptoms occur due to the suppression of natural blood cell components and the resultant anemia that causes increased development of EPO synthesis in the kidneys, leading to a significant increase in its serum level[42, 43].

Erythropoietin production is catalyzed both by anemia and low arterial oxygen compression. Therefore, it has been concluded that tissue oxygen compression regulates rythropoietin production. The increase in EPO concentration may be due to the fact that elderly people have an inadequate erythropoietin response, resulting in several consequences that may include decreased renal excretory function due to occult interstitial kidney dysfunction [44].

Conclusions

It can be concluded from the results of the present study that there were negative effects on the functions of liver and kidney as well as the serum levels of EPO and LDH in acute myeloid leukemia patients.

References

1. Chaudhari, S., Desai, J. S., Adam, A. and Mishra, P. **2014**. Jak/stat as a novel target for treatment of leukemia. *International Journal of Pharmacy and Pharmaceutical Sciences*.**6**(1): 1-7.
2. Stock, W. and Hoffman, R. **2000**. White blood cells 1: non-malignant disorders. *Lancet*,**355**(9212): 1351–1357.
3. Taylor, J., Xiao, W. and Abdel-Wahab, O. **2017**. Diagnosis and classification of hematologic malignancies on the basis of genetics. *Blood*, **130**(4), 410–423.
4. Enad, S. A. and Al-Amili, W. A. (2019). Investigation of Secondary Acute Lymphoblastic Leukemia (sALL) Among Acute Lymphoblastic Leukemia (ALL) Iraqi Patients. *Iraqi Journal of Science,* **60**(2): 223-227
5. Hoffbrand, A.V. and Moss, P.A.H. **2011**. *Essential Haematology*. 6th ed. Willey_BlackwellPublishing , Ltd. Oxford.
6. Litten, J. B., Rodriguez, M. M. and Maniaci, V. **2006**. Acute lymphoblastic leukemia presenting in fulminant hepatic failure. *Pediatr Blood Cancer*,**47**(6):842-845
7. Eugene, B., Anthony, S. B., Dennis, L.K., Stephen, L. H., Dan, L. L. and Larry, J. **2001**. *Harrison principles of internal medicine* .15th ed., New York: McGraw-Hill; pp.1711-15
8. Anderson, S. H., Richardson, P., Wendon, j., Pagliuca, A. and Portman, B. **2001**. Acute liver failure as the initial manifestation of acute leukemia. *Liver*, **21**(4):287
9. Shimizu, T., Tajiri, T., Akimaru, K., Arima, Y., Yokomuro, S. and Yoshida, H. **2006**. Cholecystitis caused by infiltration of immature myeloid cells. *Journal of Nippon Medical School*, **73**: 97-100.
10. Rasool, M., Farooq, S., Malik, A., Shaukat, A., Manan, A., Asif, M., Sani, S., Qazi, M. H., Kamal, M. A., Iqbal, Z. and Hussain, A. **2015**. Assessment of circulating biochemical markers and antioxidative status in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) patients. *Saudi journal of biological sciences*, **22**(1), 106–111.
11. Ahmed, S., Siddiqui, A. K., Rossoff, L., Sison, C. P. and Rai, K. R. **2003**. Pulmonary complications in chronic lymphocytic leukemia. *Cancer*, **98**(9):1912–1917.
12. Seki, M., Nakayama, M., Sakoh, T., Yoshitomi, R., Fukui, A.,Katafuchi, E., Tsuda, S., Nakano, T., Tsuruya, K. and Kitazono, T. **2019**. Blood urea nitrogen is independently associated with renal

outcomes in Japanese patients with stage 3-5 chronic kidney disease: a prospective observational study. *BMC nephrology*, **20**(1), 115.

13. Drent, M., Cobben, N. A., Henderson, R. F., Wouters, E. F. and van Dieijen-Visser, M. **1996**. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. *TheEuropean respiratory journal*, **9**(8):1736–1742.

14. Schumann, G., Bonora, R., Ceriotti, F., Clerc-Renaud, P., Ferrero, C. A., Ferard, G., Franck, P. F., Gella, F. J., Hoelzel, W., Jorgensen, P. J., Kanno, T. Kessner, A., Klauke, R., Kristiansen, N., Lessinger, J. M., Linsinger, T. P., Misaki, H., Panteghini, M., Pauwels, J., Schimmel, H. G. and Siekmann, L. **2002**. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. Part 3.Reference procedure for the measurement of catalytic concentration of lactate dehydrogenase. *Clinical chemistry and laboratory medicine*, **40**(6), 643–648.

15. Feng, Y., Xiong, Y., Qiao, T., Li, X., Jia, L. and Han, Y. **2018**. Lactate dehydrogenase A: A key player in carcinogenesis and potential target in cancer therapy. *Cancer medicine*, **7**(12), 6124–6136.

16. Burtis, C. A. and Ashwood, E. R. ed. **2001**. *Tietz Fundamentals of Cinical Chemistry*, 5th ed., W B Saunders Company, Philadelphia. pp 362-386

17. Henry, J. B. **2007**. *Cinical Diagnosis and Management by Laboratory Methods*. 21st ed., WB Sunders Co. pp. 258-250

18. Chateauvieux, S., Grigorakaki, C. and Morceau, F. **2011**. Erythropoietin, erythropoiesis and beyond. *Biochemical pharmacology*, **1291**, 82-303.

19. Souma, T., Suzuki, N. and Yamamoto, M. **2015**. Renal erythropoietin-producing cells in health and disease. *Frontiers in physiology*, **6**, 167.

20. Nagai, T., Yasuoka, Y., Izumi, Y., Horikawa, K., Kimura, M., Nakayama, Y. and Nonoguchi, H. **2014**. Reevaluation of erythropoietin production by the nephron. *Biochemical and Biophysical Research Communications*, **449**, 222-228.

21. Watowich, S. S. **2011**. The erythropoietin receptor: molecular structure and hematopoietic signaling pathways. Journal of investigative medicine. *the official publication of the American Federation for Clinical Research*, **59**(7): 1067–1072.

22. Morceau, F. and Diederich, M. **2012**. Breakthrough in tracking tumor erythropoietin receptor expression. *Chinese Clinical Oncology*, **11**, 1-2.

23. Alawad, M. A., Elmahdi, S. A., Ahmed, S. A. and Abdrabo, A. A. **2016**.Assessment of Liver Functions among Sudanese Leukemic Patients in Khartoum State. *Pyrex Journal of Biomedical Research* (*PJBR*),**2**(2):11-15.

24. Kumar, T. B. and Keerthana, B. **2016**. Serum cholinesterase as biomarker in liver disorders. *International journal of applied research*, **2**, 563-565.

25. Carvalhana, S., Leitao, J., Alves, A. C., Silva, A. P., Velasco, F., Medeiros, I. and Cortez-Pinto, H. **2016**. Screening for liver disease in the general population: The potential utility of using AST/ALT ratio.*Journal of Hepatology*, **64**, 498-498.

26. Verma, M., Metgud, R., Madhusudan, A. S., Verma, N., Saxena, M. and Soni, A. **2014**. A comparative study of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) levels in the saliva of diabetic and normal patients. *Biotechnic&Histochemistry : Official Publication of the Biological Stain Commission*, **89**, 529-534.

27. Lammers, W. J., Buuren, H. R., Hirschfield, G. M., Janssen, H. L. A., Invernizzi, P., Mason, A. L. and Hansen, B. E. **2014**. Levels of alkaline phosphatase and bilirubin are surrogate end points of outcomes of patients with primary biliary cirrhosis: An international follow-up study. *Gastroenterology*, **147**, 1338-1349.

28. Hassan, H. H. and Almodhaffar, A. M. J. **2014**. Incidence of Transfusion-Transmitted Viral Infections in the Haematology Department of Baghdad Teaching Hospital. *Iraqi Journal of Hematology*.**3**(1):14-22.

29. Lee, T. H., Kim, W. R. and Poterucha, J. J. **2012**. Evaluation of Elevated Liver Enzymes. *Clinics in Liver Disease*, **88**(12): 565-588.

30. Al-Kazzaz, F. F. **2011**. A Comparative Study Of Ceruleoplasmin Oxidase With Copper And Renal Function Tests In Sera Of Leukemia And Multiple Myeloma Patients During The Treatment. *Iraqi Journal of Science*, **52**(1):1-10

31. Alvarez-Lario, B. and Macarron-Vicente, J. **2010**. Uric acid and evolution. *Rheumatology (Oxford, England)*, **49**(11):2010–2015.

32. Yamauchi, T., Negoro, E., Lee, S., Takai, M., Matsuda, Y., Takagi, K., Kishi, S., Tai, K., Hosono, N., Tasaki, T., Ikegaya, S., Inai, K., Yoshida, A., Urasaki, Y., Iwasaki, H. and Ueda, T. **2013**. A high serum uric acid level is associated with poor prognosis in patients with acute myeloid leukemia. *Anticancer research*, **33**(9): 3947–3951.

33. Maiuolo, J., Oppedisano, F., Gratteri, S., Muscoli, C. and Mollace, V. **2016**. Regulation of uric acid metabolism and excretion. *International journal of cardiology*, **213**, 8–14.

34. Rasool, M., Farooq, S., Malik, A., Shaukat, A., Manan, A., Asif, M., Sani, S., Qazi, M. H., Kamal, M. A., Iqbal, Z. and Hussain, A. **2015**. Assessment of circulating biochemical markers and antioxidative status in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) patients. *Saudi journal of biological sciences*, **22**(1), 106–111.

35. Beddhu, S., Samore, M. H., Roberts, M. S., Stoddard, G. J., Pappas, L. M. and Cheung, A. K. (2003). Creatinine production, nutrition, and glomerular filtration rate estimation. Journal of the American Society of Nephrology : JASN, 14(4), 1000–1005.

36. Seller-Perez, G., Herrera-Gutierrez, M. E., Maynar-Moliner, J., Sanchez-Izquierdo-Riera, J. A., Marinho, A. and do Pico, J. L. (2013). Estimating kidney function in the critically ill patients. Critical care research and practice, 2013, 721810.

37. Elbossaty, W.F. **2017**. Lactate Dehydrogenase (LDH) as Prognostic Marker in Acute Leukemia Quantitative Method. *Journal of Blood Disorders and Transfusion*, **8**, 1-7.

38. Merza, K. S., Alaaraji, S. B. and Abdullah, B. H. (2010). Comparative study on lactate dehydrogenase, alkaline phosphatase and immunoglobulins in serum and saliva of acute leukemia and oral squamous cell carcinoma patients. *Iraqi Journal of Science,* 5**1**(2): 262-270

39. Jin, Y., Ye, X., Shao, L., Lin, B. C., He, C. X., Zhang, B. B. and Zhang, Y. P. **2013**. Serum lactic dehydrogenase strongly predicts survival in metastatic nasopharyngeal carcinoma treated with palliative chemotherapy. *European journal of cancer*,**49**(7):1619–1626.

40. Rademakers, S. E., Lok, J., van der Kogel, A. J., Bussink, J. and Kaanders, J. H. **2011**. Metabolic markers in relation to hypoxia; staining patterns and colocalization of pimonidazole, HIF-1α, CAIX, LDH-5, GLUT-1, MCT1 and MCT4.*BMC cancer*, **11**, 167.

41. Molica, S., Mirabelli, R., Molica, M., Levato, L., Mauro, F. R. and Forat, R. **2011**. Clinical relevance and treatment of nonautoimmune anemia in chronic lymphocytic leukemia. *Cancer Management and Research*, **3**, 211-217.

42. Xing, M. **2009**. Study on serum erythropoietin levels in patients of hematologic malignancies with aneamia and application of recombinant human erythropoietin. *Journal of leukemia & lymphoma*, **18**, 681-683.

43. Mohammed, S. B. **2015**. Erythropoietin level in acute leukemia. *Iraqi National Journal of Chemistry*,**15**(2):140-148.

44. Ershler, W. B., Sheng, S., McKelvey, J., Artz, A. S., Denduluri, N., Tecson, J. ,Taub, D. D., Brant, L. J., Ferrucci, L. and Longo, D. L. **2005**. Serum erythropoietin and aging: a longitudinal analysis. *Journal of the American Geriatrics Society*, **53**(8): 1360–1365.