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Estimation of some biochemical markers in acute myeloid leukemic patients before and after chemotherapy

Ayinda Mariwan Mohsen*, *Zeyan Abdullah Ali

Department of chemistry, College of Education, Salahaddin University, Erbil, Iraq.
*Corresponding author.

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Abstract

The aim of this study was to measure the levels of malondialdehyde (MDA), peroxynitrite (PN), homocysteine (HCY), and uric acid (UA) in acute myeloid leukemic patients (AML) (before and after taking chemotherapy) and compare results with a healthy control group. The present study includes 120 age ranged 18-70 years old suffered from AML as well as 40 healthy group. Subjects were collected from patients and a control group at Erbil's Nanakali Hospital, they were classified into four groups: Group 1 (G1) which includes 40 healthy subjects, Group 2 (G2) includes 40 AML patients before taking chemotherapy (newly diagnosed cases), Group 3 (G3) includes 40 AML patients after taking chemotherapy (one cycle) and Group 4 (G4) includes 40 AML patients after taking chemotherapy (more than one cycle). The results have shown a high significantly increased in levels of (MDA and UA), and significantly increased in PN in all patient groups (G1, G2, and G3) when compared to (G1). While homocysteine was a high significantly increased in (G2) when compared to (G1), and high significantly decreased in (G3 and G4) as compared to (G1). Additionally, the correlation analysis has shown that there was a significant positive correlation between malondialdehyde and peroxynitrite (r=0.4638), malondialdehyde and homocysteine (r=0.4752), and malondialdehyde with uric acid (r=0.3621). A high area under the curve of our data suggests that the determination of these parameters could be helpful to detect AML.

Keywords: Acute myeloid leukemia, homocysteine, malondialdehyde, peroxynitrite, uric acid.

Introduction

Leukemia is a kind of blood malignancy that produces cancerous white blood cells (WBCs) in the human body. The immune system may be dysfunctional or weak due to the abnormal growth of white blood cells and that influences the bone marrow and blood. They may also affect the bone marrow's capacity to produce platelet and red blood cells (RBCs) [1, 2]. Acute Myeloid Leukemia (AML) is a diverse genetic disease defined as the unregulated production, development at repose, and aggregation of immature myelogenous progenitor in the circulating bone marrow and blood, Acute myeloid leukemia is the most prevalent kind of acute leukemia in adults, making for eighty percent of overall cases of this disorder. Acute myeloid leukemia is a disorder that affected by the aged, with a median age at diagnosis of seventy years [3].

Disequilibrium between the generation of peroxides (reactive species or free radicals) and its elimination via defense systems, which include

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inhibitors (vitamins C, E, beta-carotene, Se, and L-methionine), is referred to as oxidative stress (OS) [4]. When compared with healthy cells, it has been discovered that OS causes structural alterations plus function modifications in the structure of lipids, and proteins plus (DNA and RNA) which are related to various disease cells [5].

Malondialdehyde (MDA) is a most important lipid peroxidation (LPO) product that is tumorigenic plus mutagenic [6]. Also, malondialdehyde, is a highly reliable indicator for OS [7, 8]. Superoxide (O_2•) plus nitric oxide (•NO), two free radicals, combine in a diffusion-controlled manner to generate peroxynitrite, a brief-lived and powerful abiotic oxidizer [9, 10]. Nitro tyrosine byproducts are thought of as biomarkers of peroxynitrite-induced tissue injury and have been linked to the aging of tissues. Peroxynitrite diffuses easily all over the cellular membrane, and will also decay lipids, L-methionine byproducts, L-tyrosine in polypeptides, and Genetic material to nitro guanine, and it performs as an oxidizing agent [11].

Antioxidants will be divided into enzymatic plus non-enzymatic types based on their action. non-enzyme antioxidants prevent free radical chain reactions, and enzyme-based antioxidants decompose plus eliminate free radicals in multi-stage processes assisted via coenzymes [12]. As a byproduct of the decomposition of L-methionine, homocysteine (HCY) is a non-protein amino acid that contains Sulphur [13]. Homocysteine is an important biochemical marker for total wellness conditions. Although it is unclear if homocysteine is a predictor of illness or the cause of it, there is a definite biochemical relationship between increased fasting plasma concentrations of homocysteine and many pathologic conditions, such as bone health, neurological illness, kidney disorders, plus cardiovascular disease [14]. Uric acid (UA) is the by-product of purine metabolism in mammals [15, 16]. Uric acid, an oxygen radical scavenger is a very significant antioxidant that assists in preserving the balance of blood pressure & antioxidant stress [16,17]. Indeed, there is no available data on MDA, PN, HCY, and UA in sera of acute myeloid leukemic patients (after chemotherapy) and there are very few studies comparing some of these parameters in sera of AML patients before chemotherapy with the control group. Therefore the aim of this study was to assess these parameters in sera of acute myeloid leukemic patients (before and after chemotherapy) and correlate with control groups.

Materials and Methods

Patients Selection:

The present study included 120 female and male with age ranged between 18-70 years old suffered from acute myeloid leukemia (before and after taking chemotherapy) as well as 40 healthy (female and male) with age matched with patients as a control group. Blood samples were collected from all subjects enrolled in the present study. The project was performed in Erbil's Nanakali Hospital in the period between August 2021 and February 2022. Subjects were classified into four groups: Group 1 (G1) included 40 healthy subjects, Group 2 (G2) included 40 acute myeloid leukemic patients before taking chemotherapy (newly diagnosed cases), Group 3 (G3) included 40 acute myeloid leukemic patients after taking chemotherapy (one cycle) and group 4 (G4) included 40 acute myeloid leukemic patients after taking chemotherapy (more than one cycle).

Blood Sampling:

A blood sample was collected from each patient and healthy subject in a specific-tubes for each group, and serum was separated via centrifugation at 4000 rpm for ten minutes, was divided into small portions and kept frozen at -40^{o}C until further investigation. A blood sample was used for the determination of MDA, PN, HCY, and UA.

Biochemical determination:

Lipid peroxidation assay:

Malondialdehyde (MDA) level was determined upon the reaction of thiobarbituric acid (TBA) with MDA; to yield a pink color complex of MDA-TBA2 product that is measured spectrophotometrically at 532 nm [18, 19].

Peroxynitrite (ONOO•–) assay:

The quantity of nitrophenol is equal to the quantity of peroxynitrite radical that is available in serum, and peroxynitrite ($ONOO^{\cdot}$) radical

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estimation was based on the nitration of phenol via peroxynitrite radical, which resulted in the creation of nitrophenol, the absorption was recorded at 412 nm [20].

Homocysteine (HCY) assay:

Homocysteine level was estimated by using the Human (HCY) ELISA Kit (SUN LONG Biotech Co., LTD, China) and Elisa and Elisa (Biotek, USA).

Uric acid assay:

The uric acid level was estimated by enzymatic colorimetric assay at wavelength 546 nm, by using the (CliniChem) Uric acid biolis kit, and (Biolis50i Tokyo boeki medisys). The principle of uric acid determination is the formation of Allantoine, which was formed with CO_2 and H_2O_2 from the reaction of uric acid with $2H_2O$ and O_2 by using uricase enzyme, then H_2O_2 can be oxidized with p-hydroxybenzoate and 4-amino antipyrine by peroxidase enzyme to obtain quinone imine (purple color) and water [21].

Statistical analysis:

The software program Graph Pad-Prism (version 8) and Microsoft excel 2016 were used for data analysis. The results were explained as mean ± standard error and probability (P-value). The p-value ≤ 0.05 is regarded as significant; p-value > 0.05 is regarded as non-significant, p-value ≤ 0.001 is regarded as highly significant. An ordinary one-way ANOVA test was used for multiple comparisons between the healthy control group and patients. The area under the curve (AUC) for the diagnostic accuracy in leukemia patients was calculated using ROC curve (Receiver operating characteristic) analysis. The correlation coefficient was used for the estimation of the correlation between MDA with peroxynitrite, homocysteine, and uric acid in patient groups.

Results and discussion

In the present study, 160 female and male participated and they were split into four groups (G1:40 healthy control group, G2: 40 acute myeloid leukemic patients before taking chemotherapy (new case), G3: 40 acute myeloid leukemic patients after taking chemotherapy for one cycle, G4: 40 acute myeloid leukemic patients after taking chemotherapy for more than one cycle. The serum levels of malondialdehyde, peroxynitrite, homocysteine, and uric acid in each group are summarized in Table 1 and Fig. 1.

Table 1. Serum levels of MDA, PN, HCY, and UA in control and patient groups

Gr	MDA(µmol/L) Mean ±S.E	P	P*	PN(µmol/L) Mean ±S.E	P	P*	HCY(µmol/L) Mean ±S.E	P	P*	UA(mg/dL) Mean ±S.E	P	P*
G1	2.576 ± 0.152			17.11 ± 1.194			9.043 ± 0.207			3.825 ± 0.147		
G2	9.420 ± 0.455	H.S		40.89 ± 3.364	S		10.43 ± 0.361	H.S		9.346 ± 0.582	H.S	
G3	21.35 ± 1.478	H.S	H.S	35.77 ± 2.414	S	N.S	6.188 ± 0.133	H.S	H.S	8.631 ± 0.471	H.S	N.S
G4	16.59 ± 1.554	H.S	H.S	51.61 ± 6.323	S	N.S	7.272 ± 0.181	H.S	H.S	9.881 ± 0.703	H.S	N.S

G1: Group 1 (control Group), G2: Group 2 Patients group (before chemotherapy-new cases), G3: Group 3 (Patients group after chemotherapy- one cycle), and G4: Group 4 (Patients group after chemotherapy-more than one cycle), MDA: Malondialdehyde, PN: Peroxynitrite, HCY: Homocysteine, and UA: Uric acid, P: Probability between G1 and G2, G3, G4, P*: Probability between G2 and G3, G4, S: Significant, N.S: Non-Significant, H.S: High Significant.

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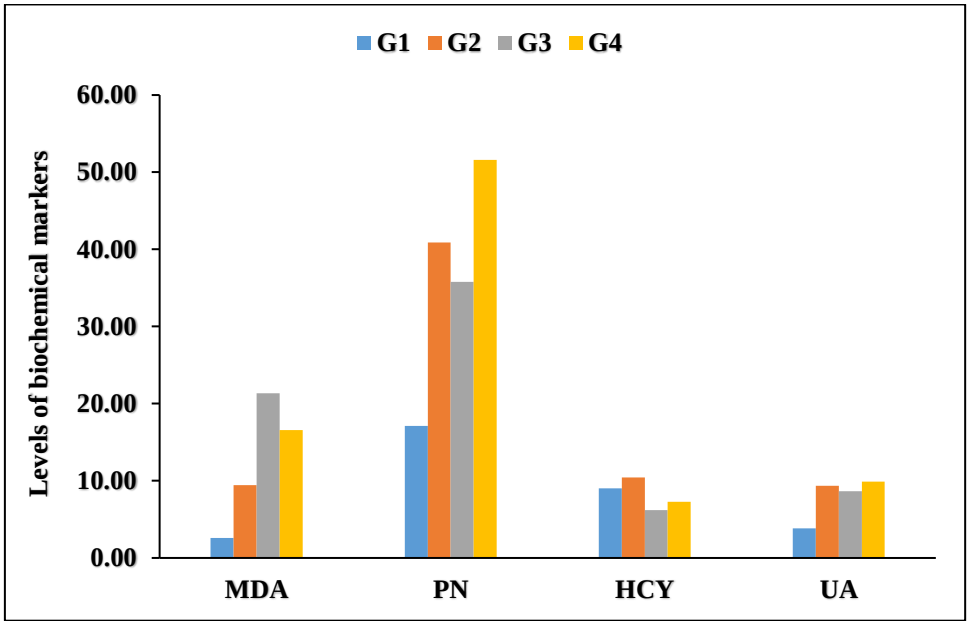

Figure 1. MDA, PN, HCY, and UA levels in sera of AML patients' group (before and after taking chemotherapy) with the healthy control group.

In Table 1 and Fig. 1, the levels of MDA of patients and healthy groups were shown. The mean value and S.E of malondialdehyde in sera of G1 were 2.576 ± 0.152, G2 was 9.420 ± 0.4558, G3 was 21.35 ± 1.478, and G4 was 16.59 ± 1.554. The mean value for MDA in G1 was lower than in G2, G3, and G4. There was a high significantly increased in malondialdehyde in the patients' group when compared with G1 (healthy control group). Malondialdehyde (MDA) is an end product and guide of lipid peroxidation (LP). It is extremely cytotoxic, and also performs as a tumor organizer and cocarcinogen. The increased serum concentration of MDA is attributed to a decrease in antioxidant defenses in many cancers [22]. Lipid peroxidation is speeded up via the oxidation of lipids; as an outcome, the manufacture of malondialdehyde is speeded up. Increased malondialdehyde levels serve as a sign of OS in individuals, which manifests as LP. Plasma malondialdehyde levels serve as a remarkable marker of leukemia, acting as both a prognostic and diagnostic indicator of illness progress [23].

In the present study, the higher levels of MDA could be due to raised levels of reactive oxygen species and possibly playing a crucial role in the beginning & development of tumor [24]. Also, the elevated levels of MDA in G3, and G4 were detected in comparison with G2 and this consequence was consistent with [24, 25]. This finding may be due to conditioning regimens. The observation of acute myeloid leukemic patients at various times of illness treatment revealed that OS is multiplying as an outcome of illness progressions as well as a consequence of treatment [22, 24, 25].

The same Table 1 and Fig. 1, also demonstrate a substantial difference in PN concentration between G1 and G2, G3, and G4. The mean ± S.E for G1 was 17.11 ± 1.194, G2 was 40.89 ± 3.364, G3 was 35.77 ± 2.414, and G4 was 51.61 ± 6.323. It was shown that the levels of PN in all patients significantly increased compared to the control group (G1). This finding agrees with an alike previous study that was done by Al-Wihaly et al, the reasons for high levels of PN in patients with AML may be due to the greater damage in the tissue and certain types of cancer such as colon, liver, lung, skin, and breast as a direct result of FR damage in the body [26]. Remarkably, the levels of PN in G3, and G4 were higher than in G2, this may be due to the treatment with anticancer chemotherapy. Because many anticancer chemotherapy drugs produce a high level of reactive species that lead to forming a high amount of PN in patients with AML after taking chemotherapy.

The mean ±S. E of homocysteine level for control G1 was 9.043 ± 0.207, G2 was 10.43 ± 0.361, G3 was 6.188 ± 0.133, and G4 was 7.272 ± 0.181. The homocysteine level in G1 was lower than in G2, it is level of HCY in G1 was greater than in G3, and G4. There was a high significantly

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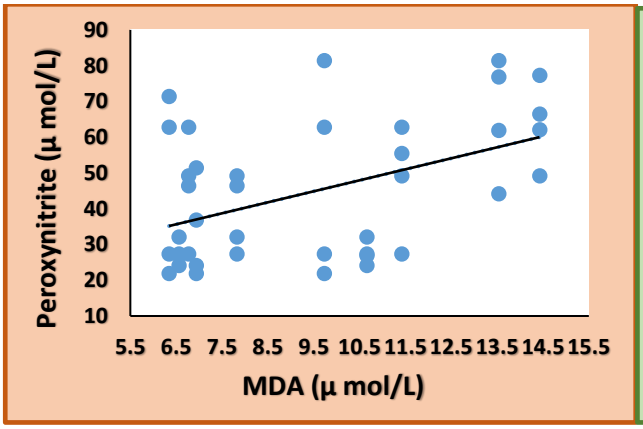
increased HCY in G2 (without chemotherapy) when compared with G1 (healthy control group). While there was a high significantly decreased HCY in G3 and G4 patients when compared with G1. Homocysteine is a sulfur-containing amino acid originating in the blood. HCY is an intermediate product in the pathway of cysteine and methionine [27]. Homocysteine is an important biochemical marker for a global wellness state, and even though it is unclear if it reflects the etiology of diseases or a marker of it, there is a strong connection between its increased fasting levels and many pathologic conditions [14]. Recently , it has been recommended that a high concentration of serum HCY is significantly associated with several diseases such as coronary artery disease, stroke, and others [28, 29]. Our finding was consistent with a recent study which has reported that there is a close relationship between tumour and hyper-homo-cysteinemia. **First**, tumor sufferers have been shown to have elevated plasma HCY levels, and venous thromboembolism is the second highest prevalent cause of mortality in tumor sufferers. **Second**, several polymorphisms in the enzymes connected to the homocysteine detoxification pathways have significant clinical associations with some malignant diseases. **Third**, there is an adverse relationship between Homocysteine and folic acid, a crucial nutrient for cell growth. **Fourth**, homocysteine has been suggested as an additional possible tumour indicator for many malignancies [30]. One of the cornerstones of medical treatment for malignancy is chemotherapeutic [31]. Regarding , AML patients after chemotherapy, the level of homocysteine is lower than the control group, this may be due to the uptake of vitamin B_6, vitamin B_{12}, and folate-rich diet especially vegetables and fruits or their supplementation [32].

The mean level of uric acid in G1 was 3.825 ± 0.147, G2 was 9.346 ± 0.582, G3 was 8.631 ± 0.471, and G4 was 9.881 ± 0.703. There was a high significantly increased in UA in patient groups G2, G3, and G4 compared with G1 (healthy control group). Our consequences are in agreement with a recent study which has shown that the levels of uric acid in acute myeloid leukemic patients before treatment were statistically significantly higher than in the control group. This serum uric acid is the outcome of the breakdown of the purine nucleic acids of leukemic cells and is an indicator of illness aggression. uric acid level decreased in G3 and increased again in G4 may be related to variety of leukemia-related problems, such as tumor lysis syndrome, medication side effects, and renal failure. According to additional research, certain patients' renal failure led to under-excretion, which contributed to the elevation in uric acid levels [16, 33].

Statistically, the correlation relationship of malondialdehyde with peroxynitrite, malondialdehyde with homocysteine, and malondialdehyde with uric acid in patient and control groups are summarized in Figs. 2A, 2B, and 2C. There was a significant positive correlation between MDA level and peroxynitrite (r=0.4638) (P-value = 0.0026), as shown in Fig. 2 A. Also, Fig. 2B has shown a statistically significant positive correlation between MDA and homocysteine (r=0.4752) (P-value = 0.0019). Fig. 2C has shown a statistically significant positive correlation between MDA and uric acid (r=0.3621) (P-value = 0.0217).



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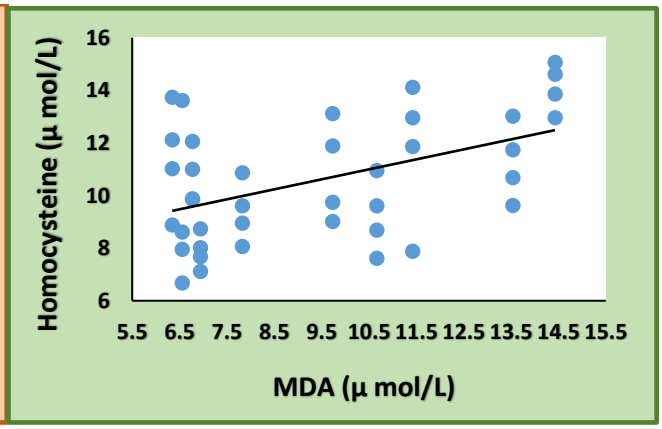

B

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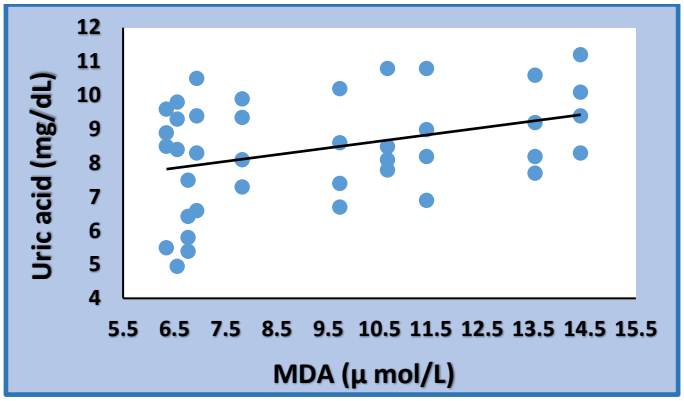

C

Figure 2. A- Correlation between malondialdehyde and peroxynitrite, B- Correlation between MDA and Homocysteine, C- Correlation between MDA and uric acid.

Figs. 3A, 3B, 3C, and 3D have displayed the receiver operating characteristic curve (ROC) (sensitivity and specificity) curve of MDA, peroxynitrite, homocysteine, and uric acid performance as a potential diagnostics biomarker for AML. A relatively high AUC (area under the curve) suggests that testing for malondialdehyde, peroxynitrite, homocysteine, and uric acid could help detect AML.

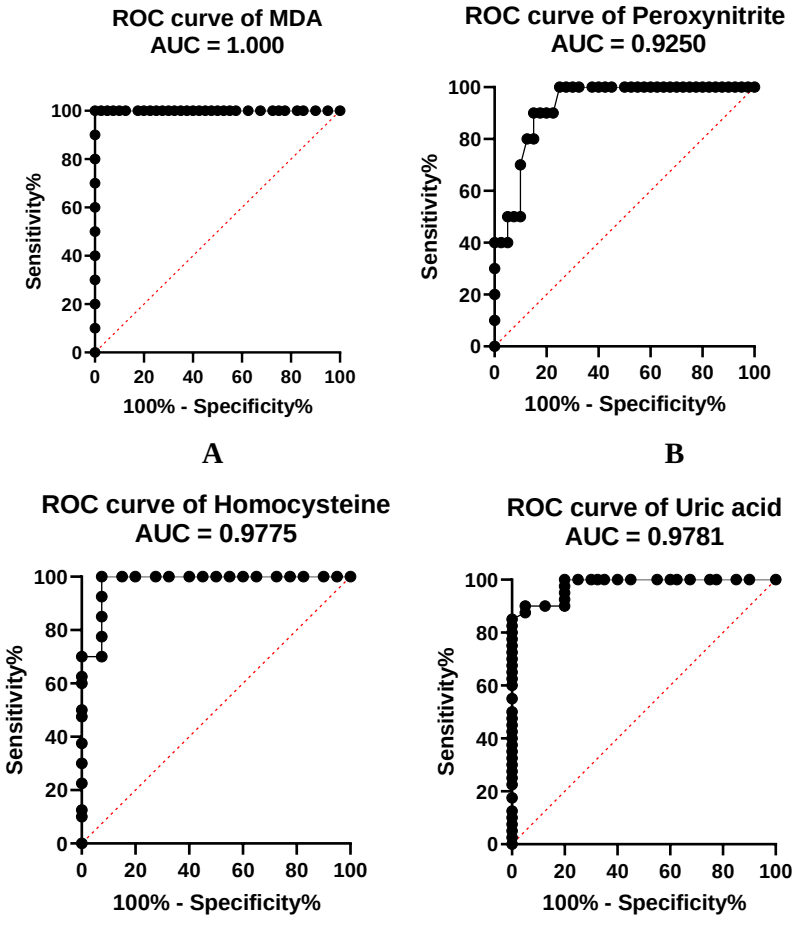

Figure. 3. ROC curve explained the sensitivity and specificity of MDA, PN, HCY, and UA for the detection of acute myeloid leukemia.

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Conclusion

Our findings suggest that was a high significantly increased in serum levels of (MDA and UA), and a significantly increased in serum concentration of PN in all patient groups when compared to the control group. While the levels of homocysteine a high significantly increased in acute myeloid leukemic patients without taking chemotherapy compared to the control group, it was a high significantly decreased in patients with AML after taking chemotherapy compared to the control group. There was a significant positive correlation between MDA level with PN, MDA with HCY, and MDA with uric acid. A high area under the curve suggests that testing for MDA, PN, HCY, and UA could help in the diagnosis of AML.

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Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Salahaddin.

Author's Contribution

A. M. M.: Collected the data, complete the practical part, statistical data analysis, write the research paper and revision the corrections. While Z. A. A.: Suggestion of the proposal project, performed interpretation of study results, revision the corrections such as supervisor.

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تقدير بعض العلامات البيوكيميائية في مرضى سرطان الدم النخاعي الحاد قبل وبعد العلاج الكيميائي

ايندە مريوان محسن، زيان عبدالله علي

قسم الكيمياء، كلية التربية، جامعة صلاح الدين، اربيل، العراق.

الخلاصة

الهدف من هذه الدراسة هي قياس مستويات كل من مالونديالديهيد (MDA) ، البيروكسينيتريت (PN) ، الهوموسيستين (HCY) وحمض اليوريك (UA) في امصال مرضى المصابين بابيضاض الدم النخاعي الحاد (AML) (قبل وبعد تناول العلاج الكيميائي) ومقارنة النتائج مع مستويات مجموعة الاصحاء. اشتملت الدراسة الحالية على 120 عينة تتراوح أعمارهم بين 18-70 سنة يعانون من (AML) اضافة الى 40 من مجموعة الاصحاء مع تطابق السن. تم جمع العينات من مرضى المصابين بسرطان الدم النخاعي الحاد ومجموعة الاصحاء في مستشفى ناناكالي في أربيل وتم تصنيفهم إلى أربع مجاميع: المجموعة 1 (G1) والتي تشمل 40 من مجموعة الاصحاء، المجموعة 2 (G2) تشمل 40 مرضى المصابين بسرطان ابيضاض الدم النخاعي الحاد قبل تناول العلاج الكيميائي (الحالات المشخصة حديثًا) ، تضم المجموعة 3 (G3) 40 مريضًا مصابا بسرطان الدم النخاعي الحاد بعد تناول العلاج الكيميائي (دورة واحدة) والمجموعة 4 (G4) تشمل 40 مريضًا مصابا بسرطان الدم النخاعي الحاد بعد تناول العلاج الكيميائي لأكثر من دورة واحدة. أظهرت النتائج ارتفاعًا معنويا في مستويات كل من (MDA و UA) ، وزيادة معنوية في مستوى PN في (G2 و G3 و G4) عند مقارنتها مع (G1) ، لكن مستوى الهوموسيستين كانت مرتفعة جدا و بشكل ملحوظ في (G2) عند مقارنتها مع (G1) ، بينما انخفض مستوى الهوموسيستين بشكل كبير في (G3 و G4) عند مقارنته مع G1. اضافة إلى ذلك ، يظهر تحليل الارتباط أن هناك ارتباطًا إيجابيًا كبيرًا بين مالونديالديهيد مع بيروكسينيتريت (r=0.4638) ، مالونديالديهيد مع الهوموسيستين (r=0.4752) ، مالونديالديهيد مع حمض اليوريك (r=0.3621). تشير المنطقة المرتفعة تحت منحنى بياناتنا إلى أن تحديد هذه الباراميترات قد يكون مفيدًا في كشف AML.

الكلمات المفتاحية: ابيضاض الدم النخاعي الحاد ، الهوموسيستين ، مالونديالديهيد ، بيروكسينيتريت ، حمض اليوريك.