The Role of miRNA-150 in Newly Diagnosed and Treated Patients with Acute Myeloid Leukemia

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ABSTRACT

Background: One form of cancer that affects the bone marrow and blood is called acute myeloid leukemia (AML). It is distinguished by the myeloblasts, or aberrant white blood cells, growing quickly and interfering with the formation of healthy blood cells. The small non-coding RNA molecule known as microRNA-150 (miR-150) is involved in the control of gene expression in several biological functions, including hematopoiesis, the process that creates new blood cells. miR-150 has been studied in relation to acute AML for its possible functions in leukemogenesis and normal hematopoiesis. **Objective:** assess the gene expression miRNA150 in AML patients.

Materials and methods: In this study, 35 patients aged between 15 to 95 years old in the pre-and post-treatment cohorts and 35 healthy volunteers were included. The samples were obtained from Medical City's Baghdad Teaching Hospital. **Results:** The age group of AML patients did not significantly differ from the control group ($P \geq 0.05$). The expression of the miR-150 gene was downregulated in the untreated AML patients while it was upregulated in the treated AML patients in comparison with the healthy control group. **Conclusions:** The analysis indicated that there was no significant difference in the age distribution between patients infected with Acute myeloid leukemia and the control group. miR-150 expression was downregulated in untreated AML patients while it was upregulated in treated AML patients. Consequently, miR-150 might be regarded as an AML prognostic marker.

Keywords: Acute Myeloid Leukemia, MicroRNA, miR-150, gene expression.
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1- Introduction

Cancer is a condition that results from genetic or epigenetic changes in somatic cells. It can spread to other body areas and has aberrant cell proliferation. Some of the various forms of cancer include sarcomas, lymphomas, carcinomas, leukemia, cancers of the central nervous system, melanoma, multiple myeloma, and other variations such as neuroendocrine and germ cell tumors (1). Leukemia has been classified into two categories: lymphoid leukemia and myeloid leukemia, depending on the kind of cell multiplying improperly (2,3). In lymphoid leukemia, the bone marrow cells that generate lymphocytes undergo a malignant change. However, the word for the cancer that appears in the cells that produce platelets, some leukocytes, and erythrocytes is myeloid leukemia. It can be acute or chronic (4) in around 1% of all new cancer cases and one-third of instances involving adult patients. An abnormal clonal population of hematopoietic stem cells that are multiplying and differentiating is usually what causes AML. When the normal maturation process of myeloid cells is disrupted, platelets (thrombocytopenia) and erythrocytes (anemia) decline, and leukocytes (leukocytosis), which are faulty myeloblasts, proliferate. Most patients die within months if therapy is not offered, mainly from hemorrhage or other disorders brought on by lowered immunity (5). miRNAs are a class of small, non-coding RNA molecules

that play a critical role in regulating gene expression in eukaryotic cells. They are roughly 22 nucleotides long and have roles in post-transcriptional regulation of gene expression and RNA silencing (6). Primary miRNAs are created by synthesizing miRNAs from DNA sequences, which are subsequently processed to provide precursor and mature miRNAs. mRNA molecules mute a gene by linking themselves to complementary sequences that either stop translation or actively promote the destruction of the target mRNA. An estimated 30% of human protein-coding genes are believed to be controlled by miRNAs (7). miRNAs are essential for the start and progression of AML (8). Humans are members of the MiR-150 family of microRNA precursors exclusive to mammals. The mature 22-nucleotide miRNA sequence from the precursor hairpin is eliminated by the enzyme Dicer. In hematopoiesis, miR-150 regulates the expression of genes supporting stem cells rather than developing into erythrocytes or megakaryocytes. In addition to miR-155, it is thought to control the differentiation of B and T cells (9). MiR-150 has been linked to several types of cancers. In osteosarcoma, it is overexpressed 50 times, and it is thought to promote the growth of cancer cells in gastric cancer. Blood plasma with the miR-150 gene present may indicate the onset of sepsis and may eventually be utilized therapeutically to treat the condition. Moreover, miR-150 is one of numerous microRNAs whose expression pattern could be used to diagnose hepatocellular carcinoma (10). The importance of miR-150 in acute myeloid leukemia (AML) has been examined in much research (8). miR-150, an essential regulator of normal hematopoiesis and carcinogenesis, has a major effect on AML. It suppresses leukemia stem cell proliferation and tumorigenicity, induces myeloid differentiation in human acute leukemia cells, and may be utilized as a biomarker to differentiate AML from controls (10, 11).

2-Materials and Methods

Study Subjects:

The study comprised 35 AML patients (categorized as 35 newly diagnosed AML patients before therapy and the same 35 subjects after therapy) and 35 healthy controls, consisting of male and female participants ranging in age from 15 to 95 years old. The patient samples were collected between November 2022 and June 2023 from the City of Medicine, Baghdad Teaching Hospital. The participants' occupation, gender, age, history of chemotherapy, use of prescription drugs for long-term illnesses, and family history of cancer were all inquired about in the questionnaire. Data was gathered using a lab assessment and a questionnaire for private interviews. Qualities for inclusion Patients: Individuals under the age of fifteen who have been diagnosed with AML. Wholesome control AML or other cancer signs are absent, and overall health appears good. They don't suffer from diabetes, thyroid problems, or any other long-term illnesses.

Blood sampling and miR-150 gene expression. A disposable 5-milliliter syringe was used to extract roughly 4 ml of venous blood from each participant group (patients and controls). After being placed in a gel tube, blood was moved to an Eppendorf tube. After that, the Eppendorf tube was kept at −20°C. TransZol Up Plus RNA Kit (Transgen Biotech, China) was used to isolate and purify RNA following the manufacturing company's recommendations. RNA was transformed to cDNA using TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix kit (Transgen Biotech, China). Expressing of miR-150 was achieved using the TransStart Green qPCR SuperMix kit (Transgen Biotech, China). The U6 gene was utilized as a control for normalization when calculating the fold change using the ΔΔCq technique (12). Table 1 lists the primers along with their respective sequences.

Table (1): The primers used in this study

Gene	Primer	Sequence (5′→3′)	Reference
Reference gene	U6Fp	CTCGCTTCGGCAGCACA	(13)
	U6Rp	AACGCTTCACGAATTTGCGT	
miRNA 150 gene	RTp	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACCACTGG	This study
	Fp	AACAGTGTCTCCCAACCCTT	
	Rp	GTCGTATCCAGTGCAGGGT	

Statistical analysis

Statistical analysis was performed using GraphPad Prism software 7.0. The probability was calculated using an unpaired t-test. For non-parametric data, the Mann-Whitney test was used. The correlation between parameters was calculated using the Spearman correlation test .

3-Results

Gene expression of miR-150 of the UP group was downregulated when compared to the HC group. On the other hand, it was upregulated in the TP group in comparison to the HC group as shown in Table (2).

Table (2): Gene expression of miR-150 and fold change of UP and TP groups compared to the HC group

Sample	U6 expression	Mir-150 expression	Δct	ΔΔct	Folding change
HC	6.109	8.638	2.529	0.000	1
UP	6.109	10.29718	4.188	1.659	0.32
Tp	6.109	8.053041	1.944	-0.585	1.5

Using receiver operating characteristic curve analysis, a cut-off value of (0.11) with an area under the curve (AUC) of (0.711) was discovered when comparing the expression levels of miRNA 150 in control and untreated patients. 64.52% was the sensitivity, and 65.52% was the specificity. Figure (1) provides an understanding of the rationale for responding to the therapy.

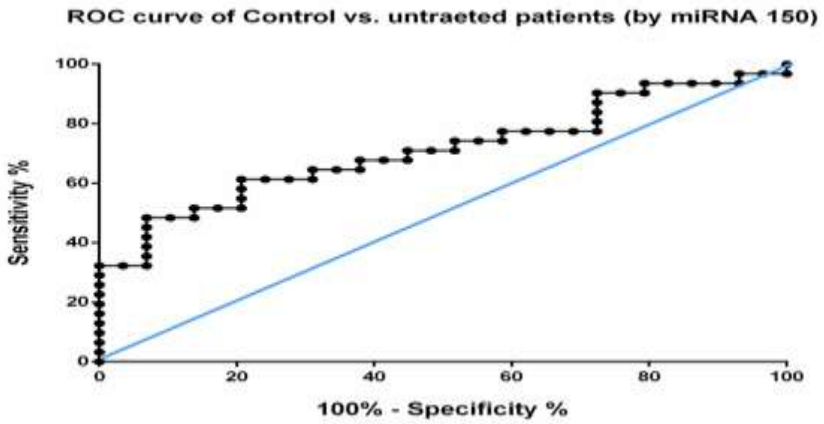

Figure (1): ROC analysis of miR-150 gene expression of the UP group compared to the HC group.

ROC analysis was used to compare the expression level of miR-150 in HC and TP groups, the cutoff value was found to be 0.248, with an AUC of 0.638. As seen in Figure (2), sensitivity (61.54) and specificity (68.97) can be helpful in diagnosis to distinguish between patients and controls.

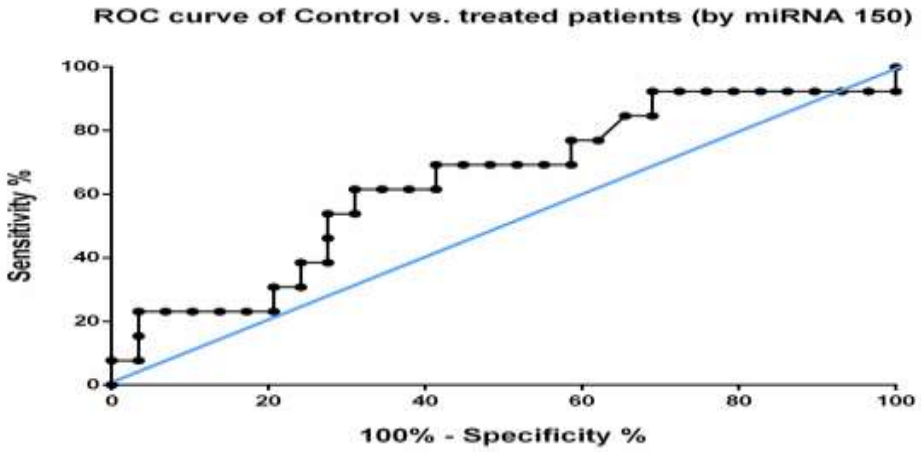

Figure (2): ROC analysis for miRNA150 gene expression of the TP group compared to the HC group.

According to the study's findings, AML patients had lower hemoglobin levels than controls. After receiving therapy, In addition, the group that had just received a diagnosis had a lower hemoglobin level than the control group. The control group had a mean±SD white blood cell count of 8.08±1.522, while the group with new diagnoses had a level of 3.538±1.105. The mean±SD values for platelets were 275.66±54.06, 37.84±9.77, and 58.31±11.34 for controls, patients, and after treatment, respectively. A p-value of 0.006 was found for each value Table (3). The current study's results corroborated those of the study, which showed significant differences in complete blood count parameters, such as hemoglobin and platelets, between AML patients and controls (P=0.001), despite the ambiguity of the WBC count result.

Table (3): White blood cells (WBC), Hemoglobin (Hb), and Platelets (PLT) displayed for each study group (HC, UP, and TP groups)

Group / Age (Year)	WBC (×10³/µL) (mean+SD)	HB (g/dl) (mean+SD)	PLT (×10³/µL) (mean+SD)
HC group	A 8.08±1.522	A 13.409±1.01	A 275.66±54.06
UP group	C 3.538±1.105	C 7.406±1.475	C 37.84±9.77
TP group	B 5.313±1.621	B 9.495±1.615	B 58.31±11.34
P-value	0.0004	0.00023	0.0006
Sign.	Sign.	Sign.	Sign.

4- Discussion

miR-150 is an important regulatory factor in hematopoiesis and cancers. MiR-150 as a diagnostic and therapeutic target may be advantageous for AML. Recent studies have indicated that miRNAs play a role in cancer, including leukemia (14). Compared to normal cells, studies on chronic amyloid leukemia (CAL) cell lines found either no miR-150 or reduced expression. MiR-150 expression governs lymphoid development, directly regulates Myloblasitosis protein, and enhances myeloid differentiation by favoring megakaryocytic differentiation over erythroid differentiation (15). AML patients had significantly lower levels of miR-150 than controls.

Downregulating miR-150 is necessary for leukemogenesis because it prevents cell transformation and serves as a tumor suppressor and gatekeeper (10). Another study found that the expression of miR-150 in AML patients and cell lines is either nonexistent or extremely low. MiR-150 targets MYB directly. In AML cell lines, cells expressing miR-150 differentiate. miR-150 promotes myeloid differentiation; low or absent miR-150 expression levels in acute leukemia cells inhibit myeloid differentiation (16). Cancer abrogates more quickly when miR-150 is overexpressed. The expression of additional cancer stem cell factors was directly reduced by miR-150. These results illuminate the specific biological functions of miR-150 in regulating the proliferation (17). In the current analysis, there were significant changes in platelet levels between patients and control participants, but not across the different categories of patients with thrombocytopenia. Low platelet counts may be the cause of bleeding in AML patients since thrombocytopenia has been commonly seen in patients receiving chemotherapy. This study supported the findings of Asif and Hassan's investigation (18) wherein the platelet levels of most patients were below 50. The patient and control groups' hemoglobin levels differed significantly; anemia was observed in every patient group, whereas the control group's hemoglobin levels did not differ significantly. It was determined by this investigation that the results were consistent with those of Al-Husseiny, who found that anemia was present in every patient without significant variance (19). There were no significant changes in HGB and PLT between the AML patient group in the current study, which included newly diagnosed and treated patients; nevertheless, there was a significant difference in WBC between patient groups. Numerous illnesses, including thrombocytopenia as well as fever, sepsis, infection, anticoagulant therapy, medications, coagulation problems, and more have all been associated with an increased risk of bleeding, including thrombocytopenia. Platelet dysfunction issues, hyperleukocytosis, or a recent bone marrow transplant**.**

5- Conclusion

Our data results show that miR-150 expression was downregulated in the AML UP group while it was upregulated in the AML TP. The analysis indicated that there was no significant difference in the age distribution between patients infected with acute myeloid leukemia and the control group.

Recommendations: Studying the correlation of the genes of this study with other hematological CML, ALL, and CLL.

References

1. Saini, A., Kumar, M., Bhatt, S., Saini, V., Malik, A. Cancer causes and treatments. International Journal of Pharmaceutical Sciences and Research, (2020); 11(7): 3121–3134.
2. Yamaguti, G.G., Lourenço, G. J., Silveira, V. S., Tone, L. G., Lopes, L. F., Lima, C. S. P. Increased risk for acute lymphoblastic leukemia in children with cytochrome P450A1 (CYP1A1)- and NAD(P)H: quinone oxidoreductase 1 (NQO1)-inherited gene variants. Acta Haematologica. (2010); 124(3): 182–184.
3. Aref, S., Azmy, E., El Ghannam, D., Haroun, M., Ibrahim, L., Sabry, M. Clinical value of CD25/CD123 co-expression in acute myeloid leukemia patients. Cancer Biomarkers: Section A of Disease Markers. (2020); 29(1): 9–16.
4. Zwaan, C. M., Reinhardt, D., Hitzler, J., Vyas, P. Acute leukemias in children with Down syndrome. Hematology/Oncology Clinics of North America. (2010); 24(1): 19–34.
5. Bagnoli, J. Analyzing Acute Myeloid Leukemia by RNA-sequencing. lmu. (2020).
6. Ardekani AM , Naeini MM. The Role of MicroRNAs in Human Diseases. Avicenna J Med Biotechnol. (2010); 2Oct; 2(4): 161-179.
7. O'Brien J, Hayder H, Zayed Y , Peng C. Overview of MicroRNA Biogene. (2018).
8. Wallace JA , O'Connell RM. MicroRNAs and acute myeloid leukemia: therapeutic implications and emerging concepts. Blood. (2017); 130(11): 1290-1301.

9. Abdelhalim DA, Elgamal BM, ElKafoury MR, Hassan NM, Hussein MM, Elhefnawi MM, Elfiky AM, Nabil M. MicroRNA-150 down Regulation in Acute Myeloid Leukaemia Patients and Its Prognostic Implication. Open Access Maced J Med Sci. (2018); 19; 6(11): 1993-2000.

10. Morris VA, Zhang A, Yang T, Stirewalt DL, Ramamurthy R, Meshinchi S, *et al.* MicroRNA-150 Expression Induces Myeloid Differentiation of Human Acute Leukemia Cells and Normal Hematopoietic Progenitors. (2013); PLoS ONE 8(9): e75815.

11. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. ature Protocols. (2008); 3: 1101108

12. Fayyad-Kazan H, Bitar N, Najar M, Lewalle P, Fayyad-Kazan M, Badran R, Hamade E, Daher A, Hussein N, ElDirani R, Berri F, Vanhamme L, Burny A, Martiat P, Rouas R and Badran B.Circulating miR-150 and miR-342 in plasma are novel potential biomarkers for acute myeloid leukemia. J Transl Med. (2013); Feb 7; 11: 31.

13. Yang M, Tang X, Wang Z, Wu X, Tang D, Wang D. miR-125 inhibits colorectal cancer proliferation and invasion by targeting TAZ. Biosci Rep. (2019); Dec 20; 39:12.

14. Wang, X., Zhu, B., Huang, Z., Chen, L., He, Z., Zhang, H. MicroRNAs as biomarkers in leukemia. Stem Cell Investigation, 1. (2014).

15. Valerie A. Morris, Taimei Yang, Paula Ladne, Jerald P. Radich, Vivian G. Oehler, MicroRNA-150 Is Down-Regulated In Chronic Myeloid Leukemia and MicroRNA-150 Expression Promotes Myeloid Differentiation., Blood. (2010); Volume 116, Issue 21, Page 1213, ISSN 0006-4971.

16. Xu, D. D., Zhou, P. J., Wang, Y., Zhang, Y., Zhang, R., Zhang, L., Wang, Y. F. (2016). miR-150 suppresses the proliferation and tumorigenicity of leukemia stem cells by targeting the nanog signaling pathway. Frontiers in Pharmacology. (2016); 7: 439.

17. Ahmed, H. S., Tahir, N. T., Obed, F. A. Cytokines profiling as prognostic markers in newly diagnosed acute myeloid leukemia. Iraqi Journal of Hematology. (2017); Volume, 6(2).

18. Asif, N., Hassan, K. Acute myeloid leukemia amongst adults. J Islamabad Med Dental College. (2013); 2: 58–63.

19. Al-Husseiny,A.H. Acute myeloid leukemia in adolescent and adult Iraqi patients clinical and haematological study. (2008); Diala J, 29: 1.

علاقة المايكرو رنا- 150 في المرضى الذين تم تشخيصهم وعلاجهم حديثًا بسرطان الدم النخاعي الحاد

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الخلاصة

خلفية البحث: سرطان الدم النخاعي الحاد هو نوع من الأورام الخبيثة التي تؤثر على الدم ونخاع العظام. ويتميز بالنمو السريع لخلايا الدم البيضاء غير الطبيعية (الخلايا النقوية) التي تتداخل مع إنتاج خلايا الدم الطبيعية. المايكرو رنا 150هو جزيء حمض نووي رايبوزي صغير غيرمشفر يلعب دورًا مهمًا في تنظيم التعبير الجيني في العمليات الخلوية المختلفة، بما في ذلك تكون الدم، وهي العملية التي يتم من خلالها تكوين خلايا الدم. تمت دراسة المايكرو رنا لأدواره المحتملة في كل من تكون الدم الطبيعي وتكوّن سرطان الدم. **الهدف:** تقييم التعبير الجيني للمايكرورنا 150 في مرضى سرطان الدم النخاعي الحاد. **المواد وطريقة العمل:** شملت هذه الدراسة 35 مريضاً قبل وبعد العلاج و35 شخصاً أصحاء تتراوح أعمارهم بين 15 و95 عاماً. تم الحصول على العينات من مستشفى بغداد التعليمي – مدينة الطب. **النتائج:** أن الفئة العمرية لم تظهر فرقاً معنوياً في مرضى سرطان الدم النخاعي المزمن مقارنة بالمجموعة السليمة ($P\geq0.05$). انخفض التعبير الجيني للمايكرو رنا 150 في المرضى غير المعالجين وازداد في المرضى المعالجين. **الاستنتاجات:** أشار التحليل إلى عدم وجود فرق كبير في التوزيع العمري بين المرضى المصابين ب بسرطان الدم النخاعي الحاد والاشخاص السليمين. التعبير الجيني ل المايكرو رنا 150 كان مرتفعا في الاشخاص المصابين بسرطان الدم النخاعي الحاد الخاضعين للعلاج بينما انخفض لدى الاشخاص غير المعالجين. وبالتالي، يمكن اعتبار المايكرو رنا 150 بمثابة علامة تشخيصية لمرض سرطان الدم النخاعي الحاد.

الكلمات المفتاحية: سرطان الدم النخاعي الحاد ، مايكرو رنا ، مايكرو رنا 150 ، التعبير الجيني.