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Study of Micro RNA 181 a3p As a Biomarker for Diagnosis of Acute Myeloid Leukemia

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ABSTRACT

Background: Tiny non-coding RNAs called microRNAs (miRNAs) are crucial for cell survival and differentiation. Numerous cancer varieties, including acute myeloid leukemia, have been linked to abnormal miRNA expression (AML). **Objective:** The objective of this study is to assess the prognostic importance of miRNA-181a-3p and its participation in the diagnosis of acute myeloid leukemia. **Subjects and methods:** Forty-five subjects were recruited from Benha University Hospital and Tanta Cancer Center. They were divided into 30 newly diagnosed adult patients with AML as patient's group and 15 apparently healthy individuals matching age and sex with patients as control group. At the beginning, blood samples (2 ml) were taken from the patients and control for complete blood picture and RT-PCR, and after 28 days of medication, more blood samples (1 ml) from the patient's group were taken in order to quantify the relative gene expression of miRNA-181a-3p. **Results:** The present study demonstrated a significant overexpression of miRNA -181a-3p gene as opposed to the control group in the AML group. Moreover, miRNA-181a-3p gene expression level decreased significantly after treatment when compared to its pretreatment level. Comparing post treatment level to control group, revealed that miRNA-181a-3p gene expression level decreased, but did not reach the control level. Non remission cases were significantly associated with higher baseline miRNA-181a-3p gene expression when compared to remitted cases. **Conclusion:** The current study revealed that miRNA-181a-3p expression level had a role in AML diagnosis and in prediction of prognosis.

Keywords: Acute myeloid leukemia, Polymorphism, Complete remission.

INTRODUCTION

The most prevalent form of leukemia in adults is acute myeloid leukemia (AML), which makes up around 80% of all cases. Ineffective erythropoiesis and bone marrow failure are generated via clonal growth of immature "blast cells" in the peripheral blood and bone marrow [(1)]. Myeloid precursor cells' failure to accomplish terminal differentiation from proliferative precursor cells into mature blood cells distinguishes AML as a highly diverse illness. AML has been linked to alterations in post-transcriptional control by miRNAs in addition to genetic and genomic aberrations, such as chromosomal translocations and inversions, gene deletions, and mutations [(2)]. AML is characterised by mutations in hematopoiesis-related genes. Even though the precise source of genetic problems is obscure, a few risk factors include smoking, chemotherapy, and radiation exposure. Aplastic anemia, paroxysmal nocturnal hemoglobinuria, myelodysplastic syndrome (MDS), and myeloproliferative maladies (MPD) can all develop into AML. Genetic alterations caused by family history should also be taken into account [(3)].

Certain RNAs regulate cells as a controlling factor, whereas others provide physiological roles. Long non-coding RNAs (lncRNAs) and short non-coding RNAs (miRNAs), which make up the largest class of non-coding RNAs (ncRNAs), are among the ncRNAs in cancer that have been the subject of the most concerned research [(4)]. Many genes' transcription can be controlled or activated by microRNAs. MiRNA abundance, subcellular localization, target messenger RNAs (mRNAs), and miRNA-mRNA interactions' affinities are only a few of the variables that affect how miRNAs interact with their target genes. Exosomes are one type of vesicle that miRNAs may be released into and used to reach target cells; another methodology includes miRNAs attaching to proteins [(5)].

AML genesis and advancement may be strongly influenced by miRNAs, which have important biological implications in hematopoietic cell differentiation and proliferation [(6)].

Four members of the MicroRNA-181 (miRNA-181) family—miRNA-181a, miRNA-181b, miRNA-181c, and miRNA-181d—have undergone extensive evolutionary conservation in nearly all vertebrate species. The genes for human miRNA-181a and miRNA-181b are situated on chromosomes 1 and 2 [(7)].

It is yet unsure how miR-181a, a member of the miR-181 family, impacts growth of cancer cells; depending on the kind of neoplasm, it may either promote or repress expansion [(8)].

The clinical application of miR-181a-3p in AML patients has not been thoroughly researched. In order to assess miR-181a-3p's diagnostic validity and forecast function in the prognosis and outcomes of AML patients, the current researchers focused at the expression of miR-181a-3p in AML patients.

SUBJECTS AND METHODS

From March 2021 to February 2022, we carried out a case-control experiment for our work. 45 individuals in all were recruited for this study from the Internal Medicine Department at Benha University Hospital and Tanta Cancer Center and they were divided into**:** Group I (patient group): contained 30 adults newly diagnosed AML and Group II (control

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group): contained 15 apparently healthy individuals matching in age and sex with patients.

Under complete aseptic conditions, 2 ml of venous blood withdrawn on EDTA tube from each subject and subsequently were divided into: One milliliters of blood for CBC (complete blood picture) and one milliliters of blood for RT-PCR.

Another one milliliters of venous blood were withdrawn at 28 days after induction of chemotherapy; were withdrawn from patient's group only for RT-PCR.

Methods:

Clinical tests: A completely automated hematology analyzer examined the complete blood picture (CBC) (Sysmex XN-L series, USA).

One milliliter of EDTA blood samples were subjected to total RNA extraction using GENEzol™ Reagent Gene aid (Taiwan), Catalogue number (cat.no) (GZR100). Reverse transcriptase was used to form complementary DNA (cDNA) using TOPscript™ DryMIX (dT18/dN6 plus), enzynomics (South Korea), (cat. no. RT220) Synthesis Kit Reverse Transcription Kits. This was done using Applied Biosystems (Veriti 96 well thermal cycler), manufacturer: life technologies – Singapore, serial number: 2990226743.

Amplification and detection of MiR-181 a3p were performed by Applied Biosystems (step one Real Time PCR) serial number 271003648 using SYBR Green with high Rox enzynomics, TOPrealTM qPCR 2X PreMIX (SYBR Green with strong ROX) (South Korea), (cat.no. RTS01S)).

That primer sequence of miRNA- 181a-3p gene: Forward 5' – 3' AGAATTACACCATCGACCGTTG. Reverse 3' – 5'TATGCTTGTTCTCGTCTCTGTGTC. Using the 2CT strategy, relative gene expression was investigated.

Ethical Approval:

The study was approved by the Ethics Board of Benha University and the patients were given all the information they need about the trial. An informed written consent was taken from each participant in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Data evaluation:

Using the Statistical Package for the Social Sciences (Armonk, New York: IBM Corp., IBM SPSS Statistics for Windows, Version 25.0), the acquired data were edited and analysed. Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Mean, Standard deviation (± SD) for parametric numerical data, while Median and range for non-parametric numerical data. Frequency and percentage of non-numerical data. The **receiver operating characteristic** Curve (ROC**)** provides a useful way to evaluate the sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups. The optimum cut off point was defined as that which maximized the **area under curve** (AUC) value **Mann Whitney Test** was used to assess the statistical significance of the difference of a non-parametric variable between two study groups. **Chi-Square test** was used to examine the relationship between two qualitative variables. **Regression analysis:** Logistic regression analysis was used for prediction of risk factors, using generalized linear models. A **p value** is considered significant if <0.05 at confidence interval 95%.

RESULTS

Our research was conducted as a case control study from March 2021 to February 2022. Clinical presentations, laboratory findings and FAB classification among studied AML patients were summarized in **table 1.**

Table (1): Clinical presentations, laboratory findings and FAB classification among studied AML patients

	Patient group
	N (%)
Fever	12(40%)
Abdominal pain	7(23.3%)
Fatigue and weakness	12(40%)
Loss of appetite and weight loss	8(26.7%)
Skin manifestations (rash, ecchymosis)	3(10%)
Bleeding per gum	10(33.3%)
Thrombotic manifestations	2(6.7%)
Pallor	20(66.7%)
Bone pain	3(10%)
Dyspnea	3(10%)
HSM	14(46.7%)
LAD	8 (26.7%)
Leucocytic count overall ($X10^9$/L)	39(18-80%)
level of hemoglobin (g/dL)	9.8(6.7-12.1%)
count of platelets ($X10^9$/L)	33.5(11-130%)
blasts in peripheral blood (%)	37.5(23-66%)
blasts in bone marrow (%)	50(30-82%)
M2	15(50%)
M3	3(10%)
M4	5(16.6%)
M5	7(23.3%)

N,number.HSM, hepatosplenomegaly. LAD, lymphadenopathy

As compared to the control group in the current investigation, the miRNA-181a-3p gene expression level increased significantly in the AML group. miRNA-181a-3p gene expression level decreased significantly after treatment when compared to pretreatment level, but did not reach the control level, with marginally significant difference between both groups. Non remission was significantly associated with higher baseline miRNA-181a-3p gene expression when compared to remitted cases **(Table 2)**.

Table (2): Comparison of MiR-181a-3p gene expression levels in AML patients and healthy controls

MiRNA-181a-3p gene expression level		**Control (number: 15)**	**Patient group (number:30)**		***P1***	***P2***		***P4***
			Before treatment	**After treatment**			***P3***	
AML	**Median (range)**	0.023 (0.0001-1)	64.951 (0.005-11431.540)	0.337 (0.0003-45.923)	**<0.0011**	<0.0577	**<0.0011**	**<0.0011**
	mean±SE	0.304± 0.109	1555.035± 614.539	7.088± 2.479				
Remission (number 12)	**Median (range)**	-	1.487 (0.057-11431.5)	0.337 (0.001-45.923)	-	-		
	mean±SE	-	905.317±649.387	5.633±2.990				
Non remission (number 18)	**Median (range)**	-	476.7 (0.005-11421.6)	1.778 (0.0003-45.318)				
	mean±SE	-	2529.613±1169.453	9.272±4.365				

P1, comparison between control and AML before treatment, using Man Whitney test
P2, comparison between control and AML after treatment, using Man Whitney test
P3, comparison between before and after treatment, using Wilcoxon test
P4, comparison between remission and non-remission, using Man Whitney test

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In the AML group, we noticed that the degree of miRNA-181a-3p gene expression did not considerably correlate with either the peripheral blast count or the marrow blast count. Moreover, no appreciable variations in its amount among several FAB categorization classes were discovered.

As per the clinical outcomes of the cases examined, full remission (CR) was attained in 12 instances (or 40%) of the cases (7 cases M2; 2 cases M5;1 case M3; 2 cases M4), while 18 cases failed to achieve CR (non-remission) (60%) (8 cases M2; 5 cases M5; 2 case M3; 3 cases M4). So, most of non-remitted cases was from M2.

Cytogenetic studies were done for 21 cases and prognostic classification was based on it. The patients were stratified according to their cytogenetic prognostic factors into Favorable, 7 cases (23.3%), intermediate, 11 cases (36.7%) and unfavorable, 3 cases (10%)**.** No significant association was found between miRNA-181a-3p gene expression level and prognostic classification based on cytogenetic analysis in AML group.

To distinguish between AML patients and control groups, the miRNA-181 a3p gene expression level underwent receiver operating characteristic (ROC) curve assessment (diagnosis of AML). The relative gene expression of miRNA-181a-3p exhibited a somewhat accurate AUC (AUC=0.843). At the optimal cutoff value for miRNA-181a-3p gene expression of 0.789, diagnosis of AML had sensitivity of 70%, specificity was 78.6%, PPV was 86.7%, NPV was 56.7%, and accuracy was 72.9% **(Figure 1).**

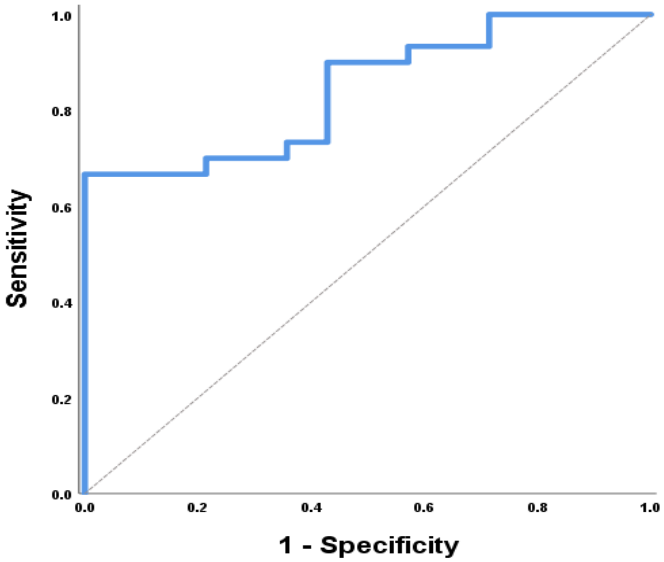

Figure (1): ROC curve of miRNA-181a-3p gene expression for diagnostic ability for patient group

Furthermore, a ROC curve analysis of the miRNA-181a-3p gene expression level was done to predict non-remission. Low accuracy AUC was observed for miRNA-181a-3p gene expression (AUC=0.681). When the optimum cutoff for miRNA-181a-3p gene expression is 34.9, prediction of non-remission had sensitivity of 77.8%, specificity of 83.3%, PPV of 87.5%, NPV of 71.4%, and accuracy of 80% **(Figure 2)**.

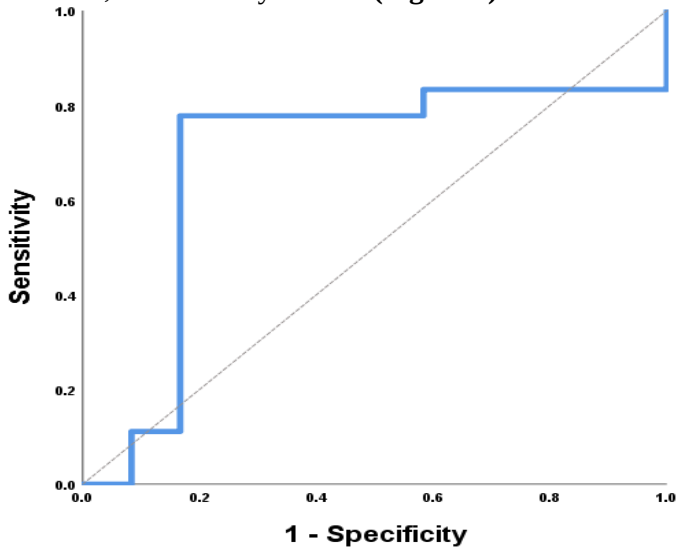

Figure (2): ROC curve of miRNA-181a-3p gene expression for all AML patients' prediction of non-remission

Age, gender, tobacco, family history of prior cancer, marrow blasts at diagnosis, intermediate risk, and miRNA-181a-3p gene expression level were used in regression analysis to predict non-remission. Older age, smoking, larger BM blasts, and miRNA-181a-3p gene expression level were linked in a univariable analysis to the likelihood of non-remission. Nevertheless, longer age, more BM blasts, and more miRNA-181a-3p0 gene expression were recognised as independent predictors of non-remission in multivariable analysis among all cases that were evaluated **(Table 3)**.

Table (3): Regression analysis for all AML cases to predict non-remission

	Univariable		Multivariable	
	P	OR(95% CI)	P	OR(95% CI)
Age	0.035	1.231(1.191-1.473)	0.032	1.293(1.123-1.493)
Gender	0.776	0.837(0.247-2.836)		
Smoking	0.025	2.100(1.582-7.579)	0.263	1.176(0.993-1.229)
Family history of pervious cancer	0.920	1.067(0.298-3.822)		
Marrow blasts at diagnosis	0.035	1.112(1.073-1.251)	0.002	1.287(1.102-1.382)
Intermediate risk (NK-AML)	0.466	1.507(0.500-4.545)		
miRNA-181a-3p gene expression	0.025	1.756(1.073-2.875)	0.028	1.847(.222-2.143)

OR, odds ratio; CI, confidence interval; NK-AML, AML with normal karyotype

DISCUSSION

One of the most prevalent forms of adult leukemia is acute myeloid leukemia (AML). It is a malady that exhibits molecular heterogeneity and is typically accompanied by poor outcomes. Based on cytogenetic and molecular abnormalities, risk categories for risk-adjusted chemotherapy are assigned to AML patients [(9)].

Even though miRNAs are engaged in the genesis and evolution of AML because of incorrect miRNA expression is linked to certain cytogenetic subgroups or gene mutations, it is probable that miRNAs might be employed as independent biomarkers to predict the fate of AML patients [(10)].

Several biologically important processes, including cell division, apoptosis, autophagy, mitochondrial function, and immunological response, are regulated by the miRNA-181 family. It's considerable that numerous studies have revealed abnormal expression of these miRNAs in the most prevalent neurodegenerative diseases (such as Alzheimer's and Parkinson's Diseases), as well as in a variety of solid tumours and hematological malignancies where they either operate effectively as oncogenic or oncomirs [(11)].

In this work, we investigated miRNA-181a-3p's clinical diagnostic relevance and function in forecasting fate and endpoints.

The present study demonstrated a significant regulated miRNA-181 a3p relative gene expression level in the AML group when compared to the control group. Moreover, miRNA-181 a3p gene expression level decreased significantly after treatment when compared to pretreatment level. Comparing post treatment level to control group, revealed that miRNA-181a-3p gene expression level decreased, but did not reach the control level, with marginally significant difference between both groups.

ROC curve revealed that miRNA-181a-3p gene expression showed moderate accuracy AUC (AUC=0.843). At miRNA-181a-3p gene expression best cut off value of 0.789, diagnosis of AML had sensitivity of 70%, specificity was 78.6%, PPV was 86.7%, NPV was 56.7%, and accuracy was 72.9%.

In keeping with this work, **Ghazimoradi *et al.*** [(12)] discovered that tumor-suppressing miRNAs are often downregulated whereas oncogenic miRNAs are frequently overexpressed in malignancies, notably leukemia. Hence, it is evident that they regulate a number of biological processes and the expression of genes, and any dysregulation of these molecules resulting in the disruption of hemostasis, which leads to pathologies like cancer.

The apoptosis-related genes are the target of miRNA-181a, which modulates apoptosis. The direct interaction between alternative apoptosis-related genes and miRNA-181a is likely to account for the enhancing/inhibiting apoptosis balance. MiRNA-181a has been demonstrated to target p53, Bax, Bcl-2, PBX3, and RalA and reduce apoptosis via interacting with protein kinase c delta (PRKCD), ATM, and Bim. This effect is shown most prominently in malignant cancerous cells [(13)].

MiRNA-181a may serve as an oncomir by speeding the G1/S transition by downregulating p27, according to one theory. **Su *et al.*** [(14)] revealed mechanisms by which PRKCD, Ca/calmodulin-dependent protein kinase kinase 1 (CAMKK1), and C-terminal domain small phosphatase 1 (CTDSP1) mRNAs, which are crucial mRNAs in regulating the cell life cycle, were directly targeted and negatively policed by MiR-181a to block granulocytic and macrophage-like differentiation. By blocking distal differentiation in both cultured HL-60 cells and $CD34^+$ hematopoietic stem/progenitor cells, miR-181a promotes a proliferative state (HSPCs).

Two mature strands, miRNA-181a-3p and miRNA-181a-5p, can be manufactured from the precursor miRNA-181a. Extracellular vesicles (R-EVs) generated from RPMI8226 cells have substantial expression of miRNA-181a-3p, which controls cellular proliferation. Targeting nuclear factor-Kabba essential modulator (NEMO/IKBKG), miRNA-181a-3p limits nuclear factor kappa (NF-B) signalling. As this route promotes the development of myeloid cells, NF-B, a key transcription factor, plays a critical cancer-promoting impact in acute myeloid leukemia (AML), and its blockage promotes the pathogenesis of AML [(15)].

12 miRNAs were found to be expressed at elevated amounts in AML samples compared to healthy controls by **Lee *et al.*** [(16)]. Of these 12 miRNAs, miRNA-181, miRNA-221, and miRNA-3154 were shown to be considerably more abundant at the time of the first AML diagnosis (before to medication) than they were after the patient had reached full remission (after chemotherapy).

Our findings confirmed that of **Qiang *et al.*** [(17)], who tested the expression of miRNA-181a-3p in AML cases against healthy controls and discovered that it is considerably elevated in AML patients and may be utilised to distinguish AML patients from controls. Moreover, it dramatically decreased in expression level in those who experienced full response post induction chemotherapy.

Ma [(18)] also observed that patients who experienced a full response to induction chemotherapy had considerably lower expression of the miRNA-181a-3p gene.

Expression levels of miRNA-181a were considerably higher in ALL patients, as per **Shafik *et al.*** [(19)]. Hence, miRNA-181a may have a function in ALL as an onco-miRNA.

Receiver operating characteristic (ROC) curves for the prediction of non-remission were developed in order to evaluate the clinical relevance of miRNA-181a-3p gene expression level in AML patients. These curves showed poor accuracy AUC (AUC=0.681). The prediction of non-remission exhibited sensitivity of 77.8%, specificity of 83.3%, PPV of 87.5%, NPV of 71.4%, and accuracy of 80% at the miRNA-181a-3p

gene expression best cut off value of 34.9. As compared to remitted cases, non-remission was substantially linked with greater baseline miRNA-181a-3p gene expression.

It was also discovered in our study that the majority of non-remitted cases came from M2. **Su *et al.*** [14] demonstrated that the M1, M2, and M3 subtypes clearly expressed higher levels of all miRNA-181 family members.

Contrasting with us, **Butrym *et al.*** [20] claimed that the clinical outcomes of patients were unaffected by the change in miRNA-181 expression after chemotherapy and that patients with greater miRNA-181 expression at diagnosis had a better prognosis than those with lower miRNA-181 expression.

Older age, smoking, larger BM blasts, and high levels of miRNA-181a-3p gene expression were linked with risk of non-remission in univariable analysis, which was used in this investigation to predict the determinants of non-remission in our study group.

CONCLUSION

AML miRNA-181a-3p can be utilised as a disease marker and to predict how well chemotherapy would affect for a certain patient.

DECLARATIONS

- **Consent for publication:** I attest that all authors have agreed to submit the work.
- **Availability of data and material:** Available
- **Competing interests:** None
- **Funding:** No fund
- **Conflicts of interest:** no conflicts of interest.

REFERENCES

1. **Vakiti A, Anastasopoulou C, Mewawalla P (2022):** Malignancy-Related Hypercalcemia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482423.
2. **Liao Q, Wang B, Li X *et al.* (2017):** miRNAs in acute myeloid leukemia. Oncotarget, 8(2): 3666-3682. doi: 10.18632/oncotarget.12343.
3. **Mundt K, Dell L, Boffetta P *et al.* (2021):** The importance of evaluating specific myeloid malignancies in epidemiological studies of environmental carcinogens. BMC Cancer, 21 (1): 227. doi: 10.1186/s12885-021-07908-3.
4. **Deogharia M, Gurha P (2022):** The guiding" principles of noncoding RNA function. Wiley Interdiscip. Rev. RNA., 13(4): e1704.
5. **O'Brie J, Hayder H, Zayed Y *et al.* (2018):** Overview of microRNA biogenesis, mechanisms of actions, and circulation. https://doi.org/10.3389/fendo.2018.00402.
6. **Wallace J, O'Connell R (2017):** MicroRNAs and acute myeloid leukemia: therapeutic implications and emerging concepts. Blood, 130(11): 1290-1301.
7. **Weng H, Lal K, Yang F *et al.* (2015):** The pathological role and prognostic impact of miR-181 in acute myeloid leukemia. Cancer Genet., 208(5):225-229. doi: 10.1016/j.cancergen .
8. **Roth E, Cao J (2015):** MiR-181 suppresses metastasis via MMP-14. Aging, 7(10): 740–741. doi: 10.18632/aging.100824.
9. **Coombs C, Tallman M, Levine R (2016):** Molecular therapy for acute myeloid leukaemia. Nature reviews Clinical oncology, 13(5): 305–318. doi: 10.1038/nrclinonc.2015.210.
10. **De Leeuw D, Verhagen H, Denkers F *et al.* (2016):** MicroRNA-551b is highly expressed in hematopoietic stem cells and a biomarker for relapse and poor prognosis in acute myeloid leukemia. Leukemia, 30(3): 742-746. Htps://doi.org/10.1038/leu.2015.160.
11. **Indrieri A, Carrella S, Carotenuto P *et al.* (2020):** The pervasive role of the miR-181 family in development, neurodegeneration, and cancer. Int J Mol Sci., 21(6): 2092. doi: 10.3390/ijms21062092.
12. **Ghazimoradi M, Karimpour-Fard N, Babashah S (2023):** The promising role of non-coding RNAs as biomarkers and therapeutic targets for leukemia. Genes, 14(1): 131. https://doi.org/10.3390/genes14010131.
13. **Feng X, Zhang C, Yang Y *et al.* (2018):** Role of miR-181a in the process of apoptosis of multiple malignant tumors: A literature review. Adv Clin Exp Med., 27(2): 263-270. doi: 10.17219/acem/66842.
14. **Su R, Lin H, Zhang X *et al.* (2015):** MiR-181 family: regulators of myeloid differentiation and acute myeloid leukemia as well as potential therapeutic targets. Oncogene, 34(25): 3226-39. doi: 10.1038/onc.2014.274.
15. **Su Y, Yuan J, Zhang F *et al.* (2019):** MicroRNA-181a-5p and microRNA-181a-3p cooperatively restrict vascular inflammation and atherosclerosis. Cell death & disease, 10(5): 365. doi: 10.1038/s41419-019-1599-9.
16. **Lee Y, Kim I, Oh S *et al.* (2019):** Small RNA sequencing profiles of mir-181 and mir-221, the most relevant microRNAs in acute myeloid leukemia. Korean J Intern Med., 34(1): 178-183. doi: 10.3904/kjim.102.
17. **Qiang P, Pan Q, Fang C *et al.* (2020):** MicroRNA-181a-3p as a diagnostic and prognostic biomarker for acute myeloid leukemia. Mediterr J Hematol Infect Dis., 12(1): e2020012. doi: 10.4084/MJHID.2020.012.
18. **Ma X (2020):** MicroRNA-181a-3p as a diagnostic and prognostic biomarker for acute myeloid leukemia, Mediterranean Journal of Hematology and Infectious Diseases, 12(1): e2020012. doi: 10.4084/mjhid.
19. **Shafik R, Abd El Wahab N, Mokhtar M *et al.* (2020):** Expression of microRNA-181a and microRNA-196b in Egyptian pediatric acute lymphoblastic leukemia. Asian Pac J Cancer Prev., 21(11): 3429-3434. doi: 10.31557/APJCP.2020.21.11.3429.
20. **Butrym A, Rybka J, Baczyńska D *et al.* (2016):** Expression of microRNA-181 determines response to treatment with azacitidine and predicts survival in elderly patients with acute myeloid leukaemia. Oncol Lett., 12(4): 2296-2300. doi: 10.3892/ol.2016.4970.