The Egyptian Journal of Hospital Medicine (July 2025) Vol. 100, Page 4537-4546

Study of microRNA 155 and microRNA 370 Expression in Newly Diagnosed Acute Myeloid Leukemia Patients

Yasmin A. Younis1, Samar M. Diab1, Iman A. Ahmedy1, Iman Atteya El-Tounsi1, Hadeel M. Shalaby1
1 Department of Clinical Pathology, Faculty of Medicine, Menoufia University, Menoufia, Egypt
Corresponding author: Samar Mokhtar Diab, **Phone number**: +2 01026275234, **E-mail**: dr.samar.mokhtar1991@gmail.com

ABSTRACT
Background: Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy marked by the clonal expansion of immature myeloid cells in the bone marrow and peripheral circulation. Gene alterations, including those affecting microRNAs (miRNAs), contribute to this process by modifying the expression of key genes. Given the essential role of miRNAs in cellular functions like growth and differentiation, their study is critical for understanding AML. **Objectives:** This investigation explored the roles of microRNA-155 (miR-155) and microRNA-370 (miR-370) in acute myeloid leukemia (AML) patients. **Methods**: The study included 50 subjects, divided into two equal groups: newly diagnosed AML patients and healthy controls. Patients underwent comprehensive diagnostic evaluations including CBC, blood film, bone marrow aspiration, and immunophenotyping. The gene expression levels of miR-155 and miR-370 were measured in all subjects using real-time PCR.
Results: AML patients exhibited significantly elevated levels of both miR-155 and miR-370 in comparison with the control group. A significant increase in miR-155, but not miR-370, was found in deceased patients compared to those who survived. ROC curve analysis confirmed the diagnostic validity of both miRNAs for AML.
Conclusion: MiR-155 and miR-370 were found to be markedly upregulated in AML patients, suggesting their significant value as diagnostic and prognostic biomarkers for the disease.
Keywords: microRNA 155, microRNA 370, expression, newly diagnosed, Acute Myeloid Leukemia.

INTRODUCTION

Acute myeloid leukemia (AML) is a clonal hematological malignancy defined by the abnormal proliferation and arrested differentiation of myeloid progenitor cells within the bone marrow [(1)]. This disruption in normal hematopoiesis results in the accumulation of immature blasts that interfere with the production of functional blood cells, ultimately leading to clinical manifestations such as anemia, recurrent infections, and bleeding tendencies. From a pathological perspective, AML is highly heterogeneous, encompassing a wide range of genetic and cytogenetic abnormalities that not only drive leukemogenesis but also influence disease behavior, treatment response, and prognosis. Epidemiologically, AML is recognized as the most common form of acute leukemia among adults, with incidence increasing markedly with age. The median age at diagnosis is approximately 65–70 years, and the disease is relatively rare in children and young adults. Importantly, despite advances in diagnostic methods and therapeutic strategies, AML continues to pose significant clinical challenges. In contrast to other leukemic subtypes, AML is associated with the highest incidence of mortality and substantially lower long-term survival rates [(2)]. Standard chemotherapy regimens can induce remission in many patients; however, relapse is frequent, and outcomes remain particularly poor in elderly individuals and in those harboring adverse genetic alterations.

The heterogeneity of AML is partly driven by a variety of genetic mutations that alter the epigenetic landscape. These mutations occur in genes with established roles in regulating chromatin and/or methylation states in hematopoietic cells, thereby altering the epigenetic pattern crucial for myeloid cell differentiation [(3)]. This aberrant epigenetic remodeling contributes to the uncontrolled expansion of leukemic blast cells. MicroRNAs (miRNAs) are a class of evolutionary conserved, short, non-coding, single-stranded RNAs, typically 19 to 22 nucleotides in length, that function as key post-transcriptional regulators of gene expression by controlling mRNA stability and translation. These molecules play an essential role in a multitude of critical biological functions, including cell growth, proliferation, differentiation, and apoptosis. In a disease context, miRNAs can function as either oncogenes or tumor suppressors, and their dysregulation has been shown to contribute to malignant transformation in both solid and hematological tumors, including AML [(4)].

The expression of miRNAs is frequently deregulated in AML through a variety of mechanisms. These mechanisms include (i) copy number alterations, which lead to a change in the dosage of miRNA genes; (ii) epigenetic changes, such as aberrant DNA methylation, that silence or activate miRNA promoters; (iii) the localization of a miRNA to an oncogenic genomic region due to chromosomal translocation or the overexpression of a nearby protein-coding gene; (iv) the aberrant targeting of miRNA promoter regions by altered transcription factors; and finally, (v) a deregulation of the miRNA processing machinery [(5)].

Within the wide spectrum of microRNAs that have been implicated in oncogenesis, miR-155 and miR-370 stand out as two of the most extensively studied and biologically relevant molecules. Each has been shown to play a distinct, yet at times overlapping, role in the regulation of tumor initiation, growth, and progression.

MiR-155 is encoded by the MIR155HG gene, which is positioned on the long arm of chromosome 21

Received: 02/05/2025
Accepted: 04/07/2025

at band 21q21.3 [6]. Under normal physiological circumstances, its transcription is activated in hematopoietic stem cells and in early myeloid progenitor populations, where it participates in maintaining immune cell homeostasis and regulating hematopoiesis. However, a substantial body of research has revealed that this microRNA is aberrantly and persistently overexpressed in a variety of malignancies, encompassing solid tumors such as thyroid, breast, colon, and lung cancers, as well as hematological neoplasms including chronic lymphocytic leukemia and several types of lymphoma. The observation that such a wide range of cancers display high miR-155 levels has led to its classification as a potent oncogenic microRNA (oncomiR) [6]. Mechanistically, miR-155 contributes to tumorigenesis through multiple pathways: it suppresses tumor-suppressor genes, enhances proliferative signaling, interferes with apoptosis, and promotes inflammatory microenvironments that are favorable to malignant transformation. Thus, miR-155 has emerged not only as a biomarker of cancer progression but also as a potential therapeutic target for interventions aiming to downregulate its expression or block its activity.

In parallel, miR-370 represents another microRNA family whose deregulation is intricately linked to cancer development. This microRNA cluster is located on chromosome 14q32.31 [7] and belongs to an imprinted region that is often disrupted in malignancies. Unlike miR-155, which shows a more uniform oncogenic role, miR-370 exerts context-dependent effects, functioning either as a tumor suppressor or as an oncogene depending on the tissue type, genetic background, and signaling pathways involved. Dysregulation of miR-370 has been observed in a wide variety of cancers, including oral squamous cell carcinoma, laryngeal carcinoma, esophageal squamous cell carcinoma, thyroid carcinoma, hepatocellular carcinoma, colon cancer, ovarian and cervical cancers, as well as bone tumors such as osteosarcoma and skin cancers such as melanoma. Experimental studies have demonstrated that in certain malignancies miR-370 downregulates oncogenic pathways, thereby acting as a protective factor, whereas in other settings it suppresses tumor-suppressor networks, favoring carcinogenesis and metastasis. This duality highlights the complex biological role of miR-370, positioning it as both a diagnostic marker and a challenging therapeutic target [7].

Taken together, the study of miR-155 and miR-370 underscores the broader concept that microRNAs can serve as critical modulators of gene expression in cancer. Their ability to act as either oncogenes or tumor suppressors emphasizes the importance of understanding the tumor-specific and microenvironment-specific contexts in which they function. Continued exploration of these molecules is expected to deepen our knowledge of cancer biology and open avenues for microRNA-based diagnostic tools and targeted therapies.

Despite the established roles of these miRNAs in various cancers, the specific effects of miR-155 and miR-370 in AML have been the subject of a few controversial articles. The existing literature presents a gap in understanding their precise expression patterns and clinical significance within this specific hematological malignancy. This study, therefore, was designed with the aim of clarifying the expression patterns of both miR-155 and miR-370, and to elucidate their clinical implications in AML. Clarifying this relationship could provide valuable insights for diagnostic and prognostic stratification, and potentially for the development of new targeted therapies.

PATIENTS AND METHODS

This study was carried out in the Clinical Pathology Department, Faculty of Medicine, Menoufia University Hospitals from January 2023 to December 2023 on fifty subjects classified into two groups: group I: patient group (25 newly diagnosed adult AML patients) and group II: control group (25 apparent healthy adult individuals). Participants were recruited from department of medical oncology at Menoufia university hospitals.

Newly diagnosed Acute Myeloid Leukemia (AML) patients were classified in accordance with the internationally recognized 5th edition of the World Health Organization (WHO) Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/ Dendritic Neoplasms [8].

The application of this standard classification system ensured the consistency and accuracy of all diagnoses within the study cohort.

Under complete aseptic conditions, 6 ml of venous blood was collected and then divided as follows: 2 ml was collected in a plain tube, and the serum was separated by centrifugation for measurement of biochemical laboratory investigations , the other 4 ml was collected into two EDTA tubes (2ml each) first EDTA tube used for CBC and blood film, and the second EDTA tube, plasma was separated by centrifugation then stored immediately at -80 c until use for miR-155 and miR-370 molecular analysis.

All patients included in the study were subjected to a comprehensive initial evaluation. This involved obtaining a complete medical history, conducting a thorough clinical examination, and performing an abdominal ultrasonography. Routine laboratory investigations were performed, including a complete blood picture, which was analyzed using a **SYSMEX** hematology analyzer from Kobe, Japan. Liver function tests, kidney function tests, and **Lactate Dehydrogenase (LDH)** levels were also measured, with the latter being quantified using an **AU680 automated chemistry analyzer** (Beckman Coulter, Japan). These standard tests were complemented by a careful examination of **Leishman-stained smears** and a bone marrow (BM) aspirate to provide a morphological assessment. Immunophenotyping of the BM aspirate was conducted using a **CytoFLEX**

benchtop Flow cytometer from Beckman Coulter Life Sciences, Japan, to identify and enumerate the different cell populations. The cytogenetic results were obtained from the patient records, and each patient's prognostic-risk group was subsequently classified based on the 2022 European LeukemiaNet (ELN) risk stratification by genetics into three distinct groups: bad, intermediate, and good prognosis [(9)].

The determination of plasma miR-155 and miR-370 was performed using **quantitative real-time PCR (qRT-PCR)**. This molecular analysis was carried out on a 7500 Fast real-time PCR system from Applied Biosystems, USA. The total RNA, including the miRNAs of interest, was extracted from **EDTA plasma samples**. Prior to extraction, the plasma samples and kit components were brought to room temperature for 30 minutes to facilitate thawing. The RNA extraction procedure was meticulously performed using the **miRNeasy Mini Kit** from Qiagen, Germany, in strict accordance with the manufacturer's instructions.

Following the extraction, the next critical step was the reverse transcription of the miRNAs into complementary DNA (cDNA). This process was executed using the **miRCURY LNA RT Kit** (Qiagen, Germany), also in precise adherence to the manufacturer's instructions. This step is essential for converting the single-stranded RNA molecules into a more stable double-stranded DNA format suitable for amplification.

The quantification of the mature miRNAs was then carried out using **qRT-PCR**. The procedure for this quantitative assessment was performed using the **miRCURY LNA miRNA PCR assay Kit** from Qiagen, Germany, in strict compliance with the manufacturer's provided protocol.

For the purpose of result calculation, the data analysis proceeded through several key stages. After the completion of the qRT-PCR cycles, specific amplification of each miRNA was confirmed by analyzing the melting curves, ensuring the integrity of the results. The **Cycle threshold (Ct)** value was automatically calculated and was defined as the cycle number at which a fluorescence signal was first detected to increase above a predefined threshold. The expression data were then normalized to a reference gene, 103a, using the **ΔCt method**. This involved subtracting the Ct values of the reference gene from the Ct values of the target miRNAs for both the patient and control groups. Subsequently, the **ΔΔCt values** were calculated by subtracting the ΔCt values of the control group from the ΔCt values of the patient group. Finally, the fold change (FC) of expression, or relative quantitation for the targeted miRNAs, was calculated using the **$2^{-\Delta\Delta Ct}$ method**.

Ethical approval: Prior to inclusion in the study, written informed consent was obtained from all patients after providing them with a clear explanation of the research objectives, procedures, potential risks, and anticipated benefits. Participation was entirely voluntary, and patients were assured of their right to withdraw at any stage without any impact on their clinical care. Confidentiality of personal and clinical data was strictly maintained throughout the research process. The study protocol underwent thorough evaluation and was approved by the Research Ethics Committee of the Faculty of Medicine, Menoufia University (Approval No. IRP 1/2023 CPATH 25). All aspects of the research were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and its subsequent amendments, ensuring respect for human dignity, autonomy, and safety.

Statistical analysis

All statistical analyses were performed using SPSS software version 20. Quantitative data were expressed as mean ± standard deviation (SD) for normally distributed variables and as median with interquartile range (IQR) for non-normally distributed variables, while qualitative data were presented as frequencies and percentages. The Student's *t*-test was applied to compare normally distributed continuous variables between patients and controls, whereas the Mann–Whitney *U* test was employed for non-normally distributed variables. Categorical variables were analyzed using the Chi-square (χ^2) test. Correlations between microRNA expression and clinical or laboratory parameters were assessed using appropriate non-parametric methods. Differences in microRNA expression across prognostic groups were evaluated by the Kruskal–Wallis test. Receiver Operating Characteristic (ROC) curve analysis was used to determine the diagnostic performance and optimal cutoff values for miR-155 and miR-370, including calculation of sensitivity, specificity, and area under the curve (AUC). Furthermore, univariate and multivariate regression analyses were conducted to identify independent predictors of outcome. A two-tailed *p*-value ≤ 0.05 was considered statistically significant throughout the analysis.

RESULTS

This study included two groups: group I ; which consisted of 25 newly diagnosed AML patients (15 male and 10 females) and control group ; which consisted of 25 apparently healthy age and sex matched individuals (20 males and 5 females).(Table 1). There were significant differences regarding CBC parameters (Hb level, platelet count, peripheral blood blast percentage and WBCs count) in the both groups (Table 1). Results of patient group showed significant increase in peripheral blood blast percentage, WBCs more than 11000/mm3, ESR, LDH and CRP results ($p<0.001$); and significant decrease in Hb level and platelet count ($p<0.001$) than control group (Table 1).

Table (1): Demographic and laboratory findings in studied groups**.**

Variable	Patients (n=25)	Controls (n=25)	Test of significance	p-value
Sex Male Female	 15(60%) 10(40%)	 20(80%) 5(20%)	$\chi 2$=2.38	0.123 (NS)
Age (Years) Mean ±SD Range	 53.16 ±15.56 19-75	 52.36 ±15.09 19-75	t= 0.18	0.854 (NS)
ESR (mm) Median (IQR) Range	 92 (85-100) 75-160	 9 (7-12) 4-20	U=6.08	**<0.001***
P.B Blast (%) Median (IQR) Range	 25 (5-50) 0-90	 0 (0-0) 0-0	U=5.28	**<0.001***
Haemoglobin (mg/dl) Mean ±SD Range	 8.01 ±1.11 5.1-10.5	 14.02 ±0.81 12.5-15.7	t=21.85	**<0.001***
WBCs($X10^3/mm^3$) Median (IQR) Range	 25 (3.3-121.5) 0.88-413	 7.4 (6-8.55) 4.7-10.2	U=1.83	0.067 (NS)
WBCs ($X10^3/mm^3$) <4 4-11 >11	 6 (24%) 4 (16%) 15 (60%)	 0 (0%) 25 (100%) 0 (0%)	$\chi 2$=36.21	**<0.001***
Platelets ($X10^3/mm^3$) Median (IQR) Range	 29 (18.5-44.5) 6-179	 268 (230.5-359.5) 175-437	U=6.03	**<0.001***
LDH (U/L) Median (IQR) Range	 568 (416.25-671.5) 247-1024	 160 (145-188) 137-237	U=6.06	**<0.001***
CRP (mg/dl) Median (IQR) Range	 48 (24-67.5) 16-100	 3 (2-4) 2-5	U= 6.11	**<0.001***

*: Statistically significant, NS: Non-significant, SD: Standard deviation, IQR: Interquartile range, U: Mann-Whitney U test, t: Student t test, $\chi 2$: Chi-squared test.

The results demonstrated that the Acute Myeloid Leukemia (AML) patient group exhibited a significantly elevated expression level of both miR-155 and miR-370 in comparison to the control group. This observed difference was highly statistically significant for both genes, with a p-value of less than 0.001. The detailed quantitative data are presented comprehensively (Table 2).

Table 2: Results of molecular Analysis of miR-155 and miR-370 expression in studied groups.

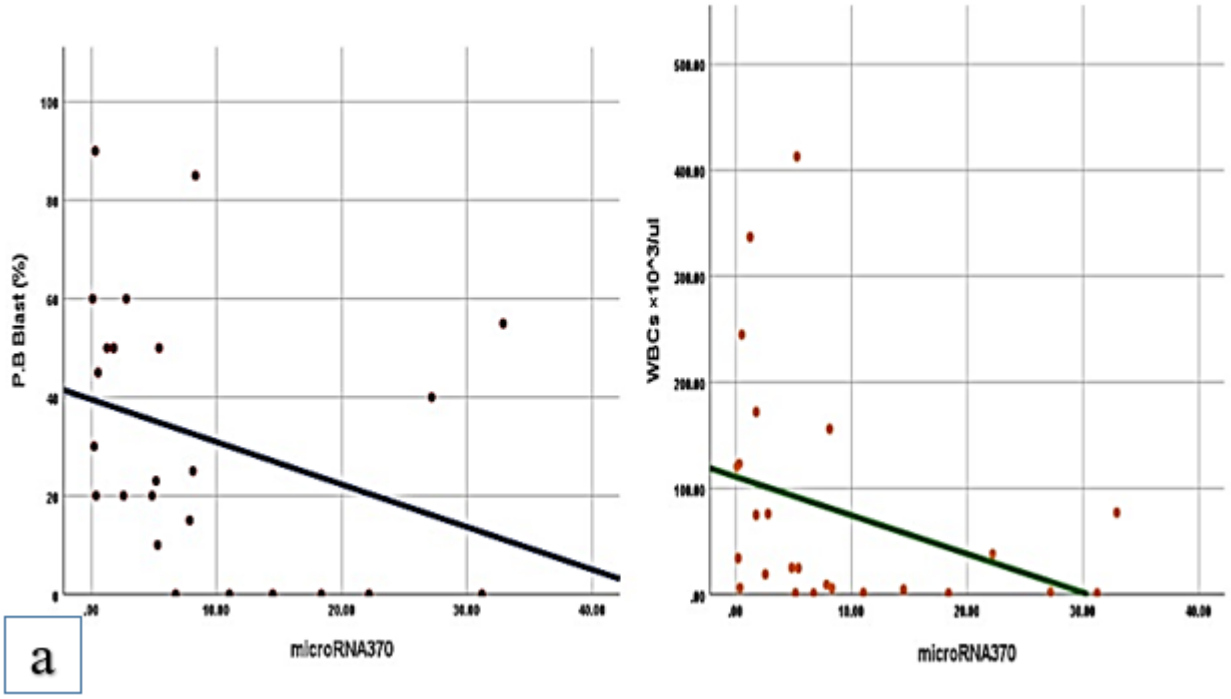
Variable	Patients	Controls	Mann-Whitney U test	P-value
miR-155 Median (IQR) Range	 9.13 (2.81-21.43) 0.58-47.98	 0.84 (0.43-1.99) 0.18-11.59	4.86	**<0.001***
miR-370 Median (IQR) Range	 5.28 (1.5-12.75) 0.09-32.9	 1.06 (0.48-2.01) 0.01-5.33	3.33	**0.001***

*: Statistically significant, IQR: Interquartile range.

There was no significant relation between miR-155 and mir-370 expression and Hepatomegaly, Splenomegaly, Hepato-splenomegaly with p-value (0.437,0.827,0.651) and (0.824, 0.511, 0.734) respectively.

There was no statistically significant relation regarding miR-155 expression and (ESR, LDH, P.B Blast percentage, Hb level, WBCs count and platelet count).

Regarding miR-370 expression; there was no statistically significant relation with (ESR, LDH, Hb level, and platelet count) while there was statistically significant relation regarding miR-370 expression and (P.B Blast percentage and WBCs count) (figure 1).



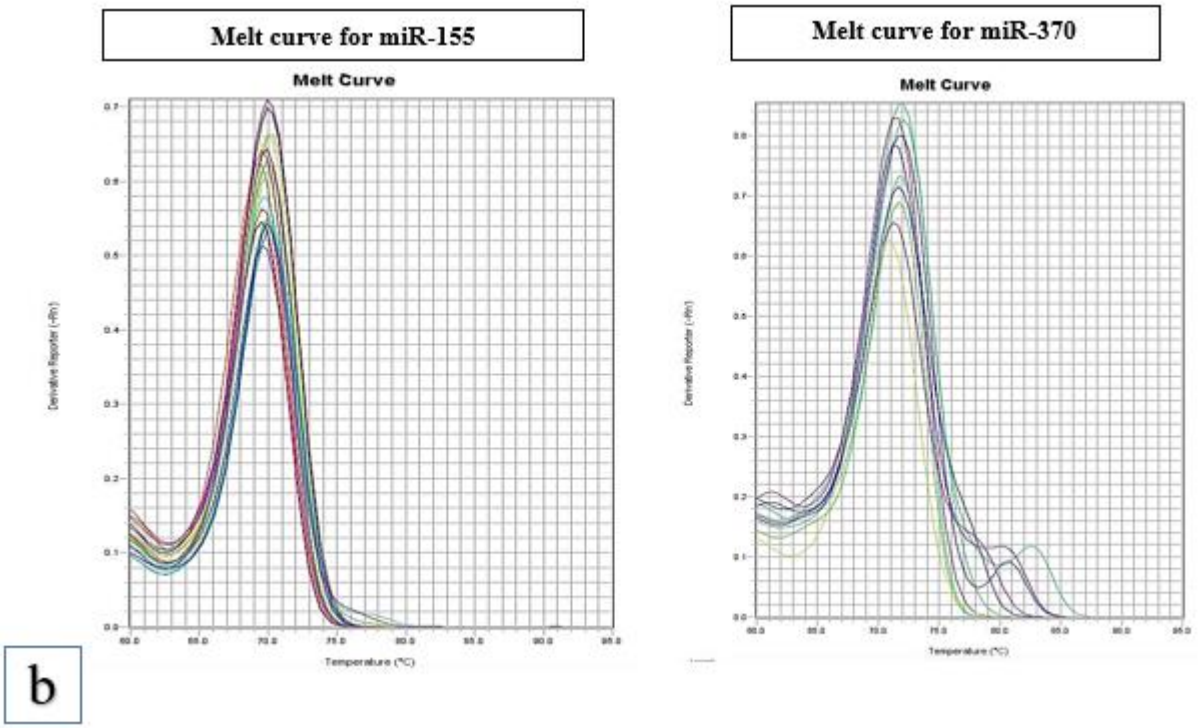


Figure (1): a) Scatter plot of miR-370 in relation to P.B blasts (%) and WBCs. B) Melt curve for miR-155 and miR-370.

The French-American-British (FAB) classification of the studied AML patient group was as the following (M0 : 1 patient (4%), M1 : 2 patients (8%), M2 : 3 patients (12%), M3 : 4 patients (16%) ,M4 : 5 patient (20%) and M5 : 10 patients) (40%) .There was no significant relation between both miRNAs (miR-155 and miR-370) expression and AML FAB classification with p-value (0.129, 0.457) respectively. There was no significant relation between (miR-155 and miR-370) expression and the following CDs (HLA-DR, CD13, CD117, Cyto MPO, CD14, CD11c). The univariate analysis revealed that these CD markers can't be used as independent predictive factors of AML. Patients with positive expression of (CD34, HLA-DR, CD117) showed worse outcome than negative ones with p-value 0.001 for all of them. While patients with double negative expression of CD34 and CD117 had better outcome with p-value 0.001. The survival rate of patients after 6 months was 24% and after 1 year was 16%. There was a significant difference between miR-155 gene expression in patients who still alive and those who died; being higher in dead patients. So that, it can be used as predictor of mortality whereas there was no significant difference between miR-370 gene expression in alive and dead patients (Table 3).

Table (3): Relation between miR-155&miR-370 expression and disease outcome in studied patients

Variable	Median (IQR)	Range	Test of significance	p-value
miR-155				
Outcome Died Alive	 12.45(4.29-28.75) 2.81(1.88-5.54)	 0.58-47.98 0.63-9.13	U=2.29	**0.022***
miR-370				
Outcome Died Alive	 5.28(0.52-14.49) 5.78 (2.36-12.68)	 0.09-32.9 1.77-27.18	U=0.26	**0.799**

*: Statistically significant, NS: Non-significant, IQR: Interquartile range, U: Mann-Whitney U test.

Also there was a statistically significant difference in miR-155 expression between different European Leukemia Net (ELN) risk classification system for AML patients with highest expression in adverse group followed by intermediate group then favorable group with p value (0.047) (Table 4).

Table (4): ELN classification in relation to miR 155 and miR-370 expression in studied patients

ELN classification	miR-155			miR-370		
	Median (IQR)	Kruskal Wallis test	p-value	Median (IQR)	Kruskal Wallis test	p-value
Favourable	2.44 (1.05-2.91)	6.12	0.047*	5,78 (3.13-7.57)	0.05	0.975
Intermediate	9.13 (4.32-25.60)			5.41 (1.14-20.83)		
Adverse	14.30 (3.96-26.99)			5.22 (0.59-16.53)		

*: Statistically significant
IQR: Interquartile range

The cut off value for miR-155 expression was 2.25 with 92% sensitivity, 84% specificity, area under the curve 0.901 and 95% CI (0.814 - 0.988) ($p < 0.001$) for discriminating patients from controls. The cut off value for miR-370 expression was 4.76 with 60% sensitivity, 96% specificity, area under the curve 0.774 and a 95% CI (0.637 - 0.912) (p 0.001) for discriminating patients from controls (Figure 2).

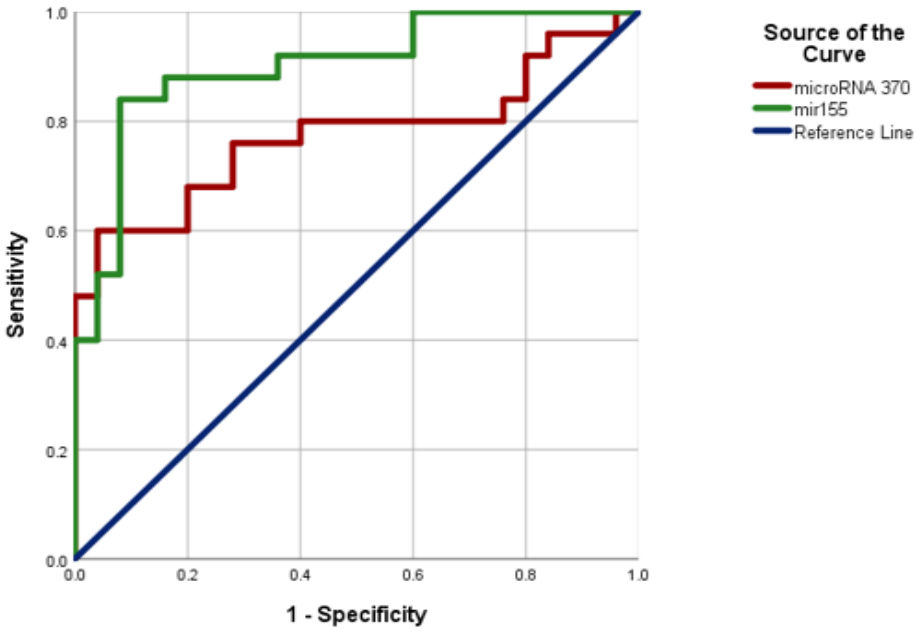


Figure (2): ROC curve of miR-155 and miR- 370 as predictors for acute myeloid leukaemia.

This multivariate analysis revealed that miRNAs (miR-155 and miR-370) are independent predictive factors of AML with 95%CI (1.118-1.956), (1.212-3.289) and p-value (0.006, 0.007) respectively.

DISCUSSION

For several decades, **Acute Myeloid Leukemia (AML)** was regarded as a fatal disease with an extremely poor prognosis. Although significant advancements in therapeutic strategies have led to some improvements in outcomes, the long-term survival rates for patients with AML continue to remain unacceptably low [(10)]. This persistent challenge is attributable to the disease's high degree of biological heterogeneity and frequent development of treatment resistance. A multitude of factors contribute to the complex pathogenesis of AML, and among these, microRNAs (miRNAs) have emerged as crucial players. These small non-coding RNAs contribute to the pathogenesis through various distinct mechanisms [(5)], including the disruption of gene expression and signaling pathways that are vital for normal hematopoiesis.

MicroRNAs (miRNAs) are fundamental regulators in a variety of key biological functions, including cellular differentiation, development, response to stress, apoptosis, and cellular growth. Their finely tuned expression is crucial for maintaining cellular homeostasis and preventing pathological states. In addition, miRNAs play vital roles in normal hematopoiesis by regulating the complex processes of hematopoietic differentiation. The aberrant expression of these small non-coding RNAs has been directly linked to the development of hematological malignancies, as their dysregulation can disrupt the delicate balance required for normal blood cell formation and maturation [(11)].

Furthermore, evidence has shown that specific miRNA expression profiles can serve as significant prognostic indicators, affecting patient outcomes and influencing their responses to standard chemotherapy regimens in acute myeloid leukemia (AML) [(12)]. Regarding specific miRNAs, miR-155 is particularly associated with myeloid cell differentiation, and alterations in its expression levels within hematopoietic stem cells have been linked to the development of myeloid disorders in the bone marrow [(13)]. The dysregulation of miR-155 can lead to a block in the differentiation process, contributing to the accumulation of immature blast cells.

The dysregulation of miR-370 is closely associated with the pathogenesis and progression of a wide range of cancers. This oncogenic or tumor-suppressive role is often mediated through its participation in several critical cellular signaling cascades, including the Wnt/β-catenin, MAPK, NF-κB, and PI3K/Akt signaling pathways [(7)]. Aberrant regulation of these pathways by miR-370 can lead to uncontrolled cell proliferation, survival, and a resistance to apoptosis, all of which are hallmarks of malignant transformation.

The results of this study revealed a significant increase in the expression of the miR-155 gene within the Acute Myeloid Leukemia (AML) cohort compared to the control group. This finding is consistent with numerous other investigations, including those by **Koolivand *et al.***, **O'Connell *et al.***, and **Xu LH *et al.***, who also documented higher expression levels of miR-155 in AML patients as compared to healthy controls [(2, 13, 14)]. This consistent observation supports its established function as an **oncomir**, a microRNA that promotes malignant transformation. The robust and reproducible elevation of miR-155 expression suggests its potential utility as a novel circulating biomarker for

the diagnosis and monitoring of AML [4]. In agreement with our findings, **Elgohary *et al.* [15]** reported that elevated miR-155 expression was markedly associated with disease relapse and mortality, as patients who relapsed or died exhibited significantly higher levels compared to those with lower expression. Similarly, another investigation demonstrated that patients classified within the adverse prognostic group displayed the highest miR-155 expression, followed by the intermediate group, whereas the favorable-risk group showed the lowest expression levels [16]. These consistent observations reinforce the notion that miR-155 overexpression correlates with unfavorable clinical outcomes and may serve as a useful prognostic biomarker in AML.

In the present study, our findings indicate that the gene expression of miR-155 was significantly higher in patients who died compared to those who survived ($p=0.022$). This suggests that miR-155 can serve as a prognostic marker for mortality in AML patients. This result is consistent with the research of Ramamurthy et al., who also reported that high miR-155 expression is an independent negative prognostic factor in pediatric AML patients, although the underlying mechanism is not yet fully understood [4,6]. Furthermore, the receiver operating characteristic (ROC) curve analysis from our study confirmed the diagnostic validity of miR-155, with a cut-off value of 2.25, a sensitivity of 92%, and a specificity of 84%, a finding that aligns with numerous other studies, including that of **Hatem *et al.* [16]**.

Our study's findings are in agreement with the results of **Elgohary *et al.***, which demonstrated that the overall survival rate was significantly higher in patients with acute myeloid leukemia (AML) who exhibited low miR-155 expression compared to those with high miR-155 expression [15]. The mechanisms leading to increased miR-155 expression are diverse, and may occur directly through the activation of key transcription factors such as NF-kB (p65) and STAT5. In FLT3/ITD AML cells, miR-155 exerts its influence on the proliferation, differentiation, and apoptosis of leukemic cells by targeting the myeloid transcription factor PU.1 [17], a master regulator of myeloid cell development. Furthermore, miR-155 also post-transcriptionally regulates SHIP1, a negative regulator of the critical PI3K/Akt pathway [18]. Given that this pathway is involved in numerous biological processes, including cell differentiation, apoptosis, transcription, and translation, the downregulation of SHIP1 by miR-155 can lead to a sustained activation of the PI3K/Akt pathway, promoting cell survival and proliferation.

In contrast to these findings, **Palma *et al.*** provided evidence for a tumor-suppressor function of miR-155 in AML cell lines. Their research showed that the overexpression of miR-155 induced myeloid maturation and apoptosis in OCI-AML3, THP1, and HL60 cell lines, a result detected using an AnnexinV/7AAD assay [19]. This apparent discrepancy is further complicated by another study that suggested increased miR-155 expression is oncogenic in FLT3 ITD cases, while in wild-type FLT3 AML cell lines, increased miR-155 may act as a tumor suppressor by promoting cell differentiation and apoptosis [19]. These observations collectively suggest that the cellular context, particularly the presence of other genomic alterations, can significantly influence the effects of miR-155 on leukemogenesis [6]. An alternative perspective posits that the differences in study results may be due to a dose-dependent role for miR-155 in AML, suggesting that the clinical and biological effects are critically dependent on the specific levels to which miR-155 is expressed [16].

Beyond its role in AML, miR-155 has been identified as a predictor of therapy response and overall survival in other hematological malignancies. For example, in Chronic Lymphocytic Leukemia (CLL), decreased plasma levels of miR-155 expression have been associated with increased overall survival, highlighting its potential as a broad prognostic biomarker [20].

Regarding miR-370 gene expression, this study revealed a statistically significant finding: the expression level of miR-370 was considerably higher in the AML group compared to the control group ($p < 0.001$). These results are in agreement with the findings of **Helbawi *et al.*** and **García-Ortí *et al.***, who explained that the overexpression of miR-370 has effects similar to the inactivation of the NF1 tumor suppressor gene [10, 11]. This suggests a leukemogenic role for miR-370 through the downregulation of NF1 in AML cells, a mechanism that can lead to uncontrolled cellular proliferation.

On the other hand, these results are not in agreement with findings from other studies, such as those by **Lin X *et al.*** or **Zhang *et al.* [21, 22]**. These alternative studies proposed that miR-370 acts as a tumor-suppressive factor by targeting multiple critical oncogenic pathways, including the transcription factor FoxM1 [21, 22]. This highlights the complex and often contradictory roles of miRNAs in cancer, which can be dependent on their specific cellular context and the pathways they regulate.

Another study that supports the tumor-suppressive effect of miR-370 was conducted by **Li *et al.* [23]**. Their research demonstrated that the long non-coding RNA (lncRNA) TUG1 can reduce the level of functional miR-370-3p, thereby facilitating the expression of MAPK1, a verified target gene of miR-370-3p. This, in turn, activates the ERK1/2 signaling pathway. The knockdown of TUG1, which results in an increase in functional miR-370-3p, significantly reduced the viability and metastasis of AML cells, while its overexpression produced the opposite effect. Our study's findings on miR-370 gene expression showed no significant relationship between living and deceased patients ($p=0.799$), suggesting it cannot be used as a prognostic marker for mortality in AML. This contrasts with the results of **Lin *et al.* [21]**, who reported that low

serum miR-370 levels may serve as a potential non-invasive prognostic marker for pediatric AML patients, as it was dramatically correlated with aggressive disease progression. However, our ROC curve analysis did reveal the validity of miR-370 as a diagnostic marker, with a cut-off of 4.76, a sensitivity of 60%, and a specificity of 96%. These findings align with **Lin *et al.*'s** [21] study, which also confirmed the diagnostic utility of miR-370 in AML patients.

Inconsistent expression levels of miR-370 have been observed in acute myeloid leukemia (AML), and the underlying mechanisms that drive these differences are not yet fully understood. It is hypothesized that miR-370 is under the regulation of a multitude of factors, and its complex interactions with numerous target genes form an intricate regulatory network. This complexity provides miR-370 with diverse biological functions that are highly dependent on the specific cellular environment and the presence of other genomic alterations [7]. The functional role of this miRNA, therefore, can vary between different subsets of AML.

The multivariate analysis performed in this study provided a significant finding: both miR-155 and miR-370 emerged as independent predictive factors for acute myeloid leukemia. These results are corroborated by other independent investigations, such as those conducted by **Ramamurthy *et al.*** and **Lin X *et al.*** [6, 21]. These researchers performed a multivariate Cox regression analysis to evaluate the impact of miR-155 and miR-370 expression levels as predictors of clinical outcome. Their findings revealed that high expression of both miR-155 and miR-370 retained prognostic significance for both overall survival and event-free survival in both adult and pediatric AML patients. This strong association highlights the potential clinical utility of these miRNAs as biomarkers for risk stratification and for guiding treatment strategies.

Patients with a positive expression of CD34, HLA-DR, and CD117 displayed a worse outcome, a finding that both aligns with and contradicts existing literature. **Demircioglu *et al.*** [24] found no correlation between CD13, CD33, CD34, CD117, and overall survival in AML patients, but noted that Cyto MPO positivity was associated with longer overall survival. In contrast, **El-Meligui *et al.*** [25] suggested that the expression of CD117 and HLA-DR could serve as prognostic markers in AML, despite their lack of association with therapy response and survival rates. The study by **Amer *et al.*** [26] proposed that CD34 is a marker for less-differentiated AML subtypes, which are associated with lower rates of complete remission, an unfavorable cytogenetic risk profile, and a worse overall outcome.

There was a significant difference in lactate dehydrogenase (LDH) levels between the AML patient group and the control group, a result that is consistent with studies supporting its use as a predictor of poor outcomes and overall survival in AML patients [27, 28].

MiR-155 and miR-370 have emerged as attractive therapeutic candidates due to their consistent overexpression across a wide range of malignancies, including hematological cancers. Several experimental strategies have been developed to counteract their oncogenic activity. For instance, the long non-coding RNA (lncRNA) CCAT1 functions as a competing endogenous RNA (ceRNA), sequestering miR-155 and thereby reducing its availability within leukemic cells, a process that influences the leukemogenic pathways in acute myeloid leukemia (AML). Although such approaches are promising, the majority remain confined to the preclinical research stage and have not yet translated into routine clinical practice [29].

In addition, the lncRNA TUG-1 has been identified as a potential therapeutic regulator of miR-370-3p. By acting as a molecular sponge, TUG-1 prevents miR-370-3p from binding to its downstream targets, thereby enhancing the malignant phenotype of AML cells through activation of the MAPK1/ERK signaling cascade. Targeting this axis is considered a promising avenue for novel treatment strategies in AML patients [23].

Beyond lncRNA-based interventions, direct inhibition of oncogenic miRNAs has also been explored. One notable example is MRG-106 (Cobomarsen), a locked nucleic acid-modified oligonucleotide specifically designed to block miR-155 activity. This agent is currently undergoing clinical evaluation to assess its safety profile, tolerability, and molecular effects in hematological malignancies [29].

CONCLUSION

The expression levels of both miR-155 and miR-370 were found to be significantly higher in adult Acute Myeloid Leukemia (AML) patients when compared to a healthy control group. This notable difference suggests that both miRNAs can be utilized as valuable diagnostic and predictive markers for AML. The analysis further revealed a distinction in their clinical utility, as miR-155 was identified as a significant prognostic marker for mortality in AML patients, whereas miR-370 did not exhibit the same prognostic value for this specific outcome.

Collectively, both miRNAs demonstrate promising potential for future clinical applications in the management of AML patients. To fully realize this potential, future research endeavors should be directed toward a deeper investigation into the factors that influence the expression of both miR-155 and miR-370. A more comprehensive understanding of the mechanisms underlying their distinct patterns of expression in AML is essential for their effective use in clinical practice and for the development of targeted therapeutic interventions.

Funding: None.
Conflicts of interest: None.

REFERENCES

1. **Döhner H, Weisdorf D, Bloomfield C (2015):** Acute myeloid leukemia. N. Engl. J. Med., 373(12):1136-1152.
2. **Koolivand M, Ansari M, Piroozian F *et al.* (2018):** Alleviating the progression of acute myeloid leukemia (AML) by sulforaphane through controlling miR-155 levels. Mol. Biol. Rep., 45(6):2491-2499.
3. **Shih A, Abdel-Wahab O, Patel J *et al.* (2012):** The role of mutations in epigenetic regulators in myeloid malignancies. Nat. Rev. Cancer, 12(9):599-612.
4. **Trino S, Lamorte D, Caivano A *et al.* (2018):** MicroRNAs as new biomarkers for diagnosis and prognosis, and as potential therapeutic targets in acute myeloid leukemia. Int. J. Mol. Sci., 19(2):460.
5. **Wallace J, O'Connell R (2017):** MicroRNAs and acute myeloid leukemia: therapeutic implications and emerging concepts. Blood, 130(11):1290-1301.
6. **Ramamurthy R, Hughes M, Morris V *et al.* (2016):** miR-155 expression and correlation with clinical outcome in pediatric AML: A report from Children's Oncology Group. Pediatr. Blood Cancer, 63(12):2096-2103.
7. **Ye L, Wang J, Yi K *et al.* (2023):** Recent findings on miR-370 expression, regulation and functions in cancer. Oncol. Rep., 49(4):78.
8. **Khoury J, Solary E, Abla O *et al.* (2022):** The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia, 36(7):1703-1719.
9. **Döhner H, Wei A, Appelbaum F *et al.* (2022):** Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood, 140(12):1345-1377.
10. **Helbawi F, Abd El H, Sherif T *et al.* (2022):** Value of microRNA 370 in acute myeloid leukemia. Egypt. J. Haematol., 47(2):88-93.
11. **Garcia-Orti L, Cristobal I, Cirauqui C *et al.* (2012):** Integration of SNP and mRNA arrays with microRNA profiling reveals that MiR-370 is upregulated and targets NF1 in acute myeloid leukemia. PLoS One, 7(10):e47717.
12. **Zebisch A, Hatzl S, Pichler M *et al.* (2016):** Therapeutic resistance in acute myeloid leukemia: the role of non-coding RNAs. Int. J. Mol. Sci., 17(12):2080.
13. **O'Connell R, Rao D, Chaudhuri A *et al.* (2008):** Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J. Exp. Med., 205(3):585-594.
14. **Xu L, Guo Y, Cen J *et al.* (2015):** Overexpressed miR-155 is associated with initial presentation and poor outcome in Chinese pediatric acute myeloid leukemia. Eur. Rev. Med. Pharmacol. Sci., 19(24):4841-4852.
15. **Elgohary T, Abu-Taleb F, Ghonaim R (2019):** The impact of miRNA-155 expression on treatment outcome in adult acute myeloid leukemia patients. J. Cancer Ther., 10(3):203-214.
16. **Hatem A, Ghonaim R, Haggag R (2021):** Prognostic impact of microRNAs (miR-155, miR-10a, let-7a) on the outcome of adult patients with acute myeloid leukemia. Zagazig Univ. Med. J., 27(5):810-825.
17. **Gerloff D, Grundler R, Wurm A *et al.* (2015):** NF-κB/STAT5/miR-155 network targets PU.1 in FLT3-ITD-driven acute myeloid leukemia. Leukemia, 29(3):535-547.
18. **Jimbu L, Mesaros O, Joldes C *et al.* (2024):** MicroRNAs associated with a bad prognosis in acute myeloid leukemia and their impact on macrophage polarization. Biomedicines, 12(1):121.
19. **Palma C, Al Sheikha D, Lim T *et al.* (2014):** MicroRNA-155 as an inducer of apoptosis and cell differentiation in acute myeloid leukaemia. Mol. Cancer, 13:79.
20. **Ferrajoli A, Shanafelt T, Ivan C *et al.* (2013):** Prognostic value of miR-155 in individuals with monoclonal B-cell lymphocytosis and patients with B chronic lymphocytic leukemia. Blood, 122(11):1891-1899.
21. **Lin X, Wang Z, Wang Y *et al.* (2015):** Serum MicroRNA-370 as a potential diagnostic and prognostic biomarker for pediatric acute myeloid leukemia. Int. J. Clin. Exp. Pathol., 8(11):14658-14666.
22. **Zhang X, Zeng J, Zhou M *et al.* (2012):** The tumor suppressive role of miRNA-370 by targeting FoxM1 in acute myeloid leukemia. Mol. Cancer, 11:56.
23. **Li G, Zheng P, Wang H *et al.* (2019):** Long non-coding RNA TUG1 modulates proliferation, migration, and invasion of acute myeloid leukemia cells via regulating miR-370-3p/MAPK1/ERK. Onco Targets Ther., 12:10375-10388.
24. **Demircioglu S, Ekinci O, Doğan A *et al.* (2022):** Prognostic significance of flow cytometric immunophenotyping in patients with acute myeloid leukemia. Bezmialem Sci., 10(4):112-135.
25. **El-Meligui Y, Abd Elrhman H, Salahuddin A *et al.* (2021):** Correlation study on HLA-DR and CD117 (c-Kit) expressions: Its prognosis and treatment response in acute myeloid leukemia patients. Pharmgenomics Pers. Med., 14:381-393.
26. **Amer A, Abdelhaleim A, Salah H (2020):** CD34 expression in adult acute myeloid leukemia is an independent poor prognostic factor. Zagazig Univ. Med. J., 26(5):823-831.
27. **Xiao Z, Gong R, Chen X *et al.* (2021):** Association between serum lactate dehydrogenase and 60-day mortality in Chinese Hakka patients with acute myeloid leukemia: A cohort study. J. Clin. Lab. Anal., 35(12):e24049.
28. **Taalab M, Shaaban Y (2023):** Prognostic impact of pretreatment serum lactate dehydrogenase levels in adult patients with acute myeloid leukemia: Single center experience. Benha Med. J., 40(3):684-692.
29. **Mahesh G, Biswas R (2019):** MicroRNA-155: a master regulator of inflammation. J. Interferon Cytokine Res., 39(6):321-330.