Role of Mir-424 Expression in Acute Myeloid Leukemia and Correlation with Nucleophosmin 1 (NPM1) Mutations

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Original Article

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ABSTRACT

Background: Acute myeloid leukemia (AML) compromises a heterogeneous group of primary hematopoietic tumors that arise from bone marrow (BM) progenitor cells. Genetic mutations lead to clonal expansion and neoplastic changes. MicroRNAs (miRNAs) are small noncoding RNAs that control numerous gene expression. These genes are implicated in hematopoietic stem/progenitor cell commitment and differentiation.

Subjects and Methods: The present study was carried out on 40 newly diagnosed patients with AML in addition to 40 matched controls. Quantitative reverse transcription real-time PCR (qRT-PCR) assay was performed to quantify miR-424. In addition, RT-PCR assays were performed to evaluate NPM1 mutations in a new Egyptian AML patient cohort and determine their correlation with miRNAs as well as their expression with demographic, clinical, and laboratory data.

Results: No statistically significant differences were detected between the mean CT values of patients and controls for Mir-424 and RNU6B. NPM1 results revealed marked differences between controls and patients. Old age and NPM gene mutation were considered as potent predictors of poor outcomes.

Conclusion: Mir-424 is not implicated in AML pathogenesis. Additionally, no correlation was found between miR-424 and NPM1,but NPM mutation was associated with poor prognosis.

Key Words: AML, miR-424, NMP, qRT-PCR.

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INTRODUCTION

AML is a BM disease caused by genetic alterations in blood cell precursors, leading to the excessive production of aberrant BM stem cells. Extramedullary manifestations like cutaneous leukemia and myelosarcoma can arise, but the fundamental cause of the disease is aberrant blood cell production. In some instances, causative factors like exposure to specific chemicals and prior chemotherapy have been determined. Most cases result from alterations in genes caused by isolated genetic mutations and chromosomal abnormalities without an apparent triggering agent[1].

The 2017 guidelines of the European LeukemiaNet (ELN) risk stratification, which incorporate genetic mutations and cytogenetic abnormalities, are commonly utilized for predicting AML prognosis[2].

Immature cells initially accumulate in the BM and then rapidly migrate to the blood and other parts of the body, including the central nervous system, liver, spleen, testes, and lymph nodes[3,4].

AML diagnosis depends on the analysis of BM and peripheral blood (PB) (including blast count and complete blood cell count). The precise diagnosis can be determined through n integrating morphologic (cytology and histology), immunophenotypic, molecular and cytogenetic data. This is in line with previous editions, with expanded numbers of disease types and subtypes that are molecularly defined. It is hoped that the genetic underpinnings of the classification will prompt the provision of health resources to ensure that the necessary genetic testing platforms are available to peruse the full potential of the classification[5].

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Extensive research has been conducted to identify prognostic biomarkers for AML, but it remains a disease with elevated mortality rates and a highly unstable prognosis. The 5-year overall survival rate is roughly fifty percent and only impacts elderly patients, with a 20% survival rate two years post-diagnosis[6,7].

The results of molecular abnormalities and cytogenetics at diagnosis are crucial prognostic factors, with elevated predictive value for overall survival, recurrence risks, disease-free survival, and complete remission rate[8,9,10].

AML etiology in the majority of cases remains undetermined. However, risk factors include chemical exposure (formaldehyde and benzene), older age, smoking, male sex, chronic myeloproliferative neoplasms (e.g., myelodysplastic syndromes (MDS and erythrocytic vera), chemotherapeutic agents (topoisomerase II inhibitors and alkylating agents), exposure to high radiation, Bloom syndrome, genetic syndromes (Fanconi anemia), and AML family history[11,12].

It is known that miRNAs are large groups of small noncoding RNAs that negatively regulate the target gene expression by binding to mRNA 3' untranslated region (UTR), inducing mRNA degradation or translational inhibition. MiRNAs significantly contribute to carcinogenesis, angiogenesis, hematopoiesis, apoptosis, proliferation, differentiation, and metastasis. In contrast, miRNAs' aberrant expression has been frequently observed in hematopoietic malignancy chemoresistance and pathogenesis[13].

The MiR-424 is crucial for regulating various vital cellular processes, including differentiation, migration, and cell proliferation[14]. Recently, MiR-424 has been categorized as part of a significant cluster family, which includes miR-15/miR-16. These members are typically recognized as tumor suppressors that promote apoptosis and hinder cell growth[15].

NPM1 is a crucial gene that encodes a nucleolar protein responsible for various functions, such as stabilizing the tumor suppressor protein p14 ARF, regulating ribosome biogenesis, and controlling centrosome duplication[16]. Nearly all mutations, totaling 50 molecular variations discovered so far, occur in exon 12 of the NPM1 gene[17], resulting in substantial alterations in the C terminus of the NPM1 protein[18].

Changes in NPM1 transport characteristics result in NPM1 variant accumulation in AML cell cytoplasm, which is believed to be crucial for leukemogenesis[19]. In addition, NPM1 mutations are considered an early event in leukemogenesis[20].

The current study aimed to determine mir-424 contribution to AML prognosis and pathogenesis to assess the correlation between both miRNAs and the NPM1 gene.

SUBJECTS AND METHODS

The study comprised two subject groups: a patient group of 40 de novo AML patients, including 21 females and 19 males. The participants' ages ranged from 20 to 70 years, with a mean age of 41.63 ± 13.26 years. In addition, the study compromised 40 age- and sex-matched controls (24 females and 16 males). The age range was between 19 and 50 years, with a mean age of 32.45 ± 7.33 years. Furthermore, AML diagnosis depended on detecting 20% or more blasts within the BM, myeloperoxidase-positive staining, and an immunophenotypic pattern consistent with an AML diagnosis.

All patients were subjected to a comprehensive medical evaluation, including a detailed medical history, thorough clinical examination, and a full range of medical tests such as Leishman-stained PB, BM smears, liver function tests, complete blood count, renal function tests, coagulation tests, and LDH.

We also performed immunophenotyping (IPT) of BM/PB samples utilizing whole blood lysis using EPICS XL flow cytometer (Coulter-USA) and a routine monoclonal antibody panel for AML. This panel includes myeloid markers (CD 13, CD33, CD 117, HLA-DR, MPO), B-cell lymphoid markers (CD 20, CD 19, and CD 10), as well as T-cell lymphoid markers (CD7, CD5, CD3, and CD2), monocyte marker (CD 14), and stem cell marker (CD 34).

A sample was deemed positive for a particular marker when at least 20% of the cells exhibited expression of this marker. Markers were deemed positive if ≥10% of cells expressed them, except for CD34 and MPO.

Real-time PCR using Light Cycle probe design software (Roche Diagnostics, GmbH, Germany) for detection and quantification of miR-424 expression levels and NPM1 mutations.

Sampling

All samples were collected after research ethics committee approval. Moreover, all subjects provided formal written consent. Data confidentiality was maintained in accordance with the revised Helsinki Bioethics Declaration.

Three ml of venous blood was collected in a sterile vacutainer and placed on ethylenediaminetetraacetic acid (EDTA). They were used to perform CBC and IPT. Three ml of BM was aspirated. A few drops were used for immediate BM testing using Leishman and MPO-stained films. One ml was collected in a heparinized tube for FISH analysis. The remaining samples were collected on EDTA for IPT.

For miRNA expression by PCR, 300-500 microns of anticoagulated PB/BM samples were added to 1.3 miRNA stabilization solution (RNAlater®) in a sterile RNase-free microcentrifuge tube. To fully penetrate the cells and

stabilize the RNA, they were stored at 4 °C overnight and then kept at −20 °C until RNA extraction. Serum samples were collected for other tests.

RT- PCR

The RiboPure™ Blood Kit from Applied Biosystems, Ambion (Cat. Nos. AM1560, AM1556) was utilized to isolate total RNA from anticoagulated blood mixed with post-processed RNA solution following the manufacturer's instructions. Total RNA was isolated from whole blood.

Two-Step RT-PCR: The TaqMan® MiRNA Reverse Transcription Kit (P/N 4366596) and TaqMan® miRNA Primers and Hybridization probes for miR-424 and RNU6B (P/N 4427975) were obtained from Applied Biosystems. They were used to detect and quantify miR-424 over a dynamic range of over 6 logs. Moreover, TaqMan® miRNA Reverse Transcription Kit compromises RT buffer, dNTPs, RNase inhibitors, and Multiscrib™ RT enzyme.

In order to normalize the results, TaqMan® miRNA Assay (2 sets) was utilized, which comprised the following: forward PCR primer, reverse PCR primer (#4366596), TaqMan® MGB probe, Taqman® Universal Master Mix (#4440043), and TaqMan® miRNA control (#4427975). For NPM1: we used a pre-built TaqMan® SNP real-time PCR assay consisting of the Pure Link DNA kit (#K182001), universal Taqman Master Mix (#4440043), and SNP Assay (#4351379).

For each 15 µL RT reaction, 1–10 ng of total RNA was combined with RT master mix at a ratio of 5 µL total RNA: 7 µL RT master mix. Then, 12.0 µL of this mixture was added to the reaction tube. Afterward, 3 µL of RT primers from each assay set were added to the corresponding RT reaction tubes, increasing the total volume per well to 15 µL. Following a brief centrifugation, the reaction vessels were sealed, placed on ice, and loaded into a thermocycler.

Data analysis: comparative CT method (ΔΔCT)

The miRNA expression level was measured utilizing the CT method. Each miRNA's expression is calculated as the CT value difference between the miRNA and the average CT value of a reference gene in a specific sample within a sample set[21]. RNU6B was utilized as the reference gene. Additionally, The relative fold change of each candidate miRNA (within each group) was determined utilizing the 2-(ΔΔCT)[22].

The ΔCT of each miRNA in each sample was calculated as follows: ΔCTsample = CT target miRNA – CT RNU6B.

Then, ΔΔCT was determined as follows: ΔΔCT = (CTmiRNA – CRNU6B) patient − (CT miRNA – CRNU6B) control.

Statistical methods

Statistical analysis was done utilizing the 21st version of the SPSS software (SPSS Inc., Chicago, IL, USA). Quantitative data was described in the form of mean ± standard deviation (mean ± SD), or range (median, if appropriate). Qualitative data were described as percentages and numbers. For analysis statistics, the following tests were used: Fisher's exact test, Student's t-test, Pearson's chi-square test, and Mann-Whitney test to compare two median values.

Patients with expression values higher than the median of all samples were categorized as demonstrating upregulated mir-424 expression. Correlations between various variables were calculated utilizing the Pearson correlation coefficient for linear relationships. P-values below 0.05 are deemed statistically significant, while those below 0.01 are considered highly statistically significant.

RESULTS

Forty patients diagnosed with de novo AML were included in the study and selected as the patient group. Patients were recruited from the Department of Medical Oncology at Kasr al-Aini Faculty of Medicine, Cairo University, and the National Cancer Institute. A control group consisting of 40 healthy volunteers of the matched age and sex was also included.

Regarding the demographic data of patients and controls, there were no substantial differences between the two groups investigated in terms of age (p-value = 0. 06) and sex (*p-value*)=0.326).

Regarding clinical data, weight loss was reported in 32.5% of cases, fever/infection in 40% of cases, bleeding tendency in 27.5%, hepatomegaly in 30%, splenomegaly in 55% of cases, and splenomegaly in 15% of cases.

Regarding the laboratory data of patients, the total white blood cell count ranged from 12. 40 to 153.00 x 109/L (median = 34.75). Hemoglobin levels ranged from 3.40 to 13.40 Hb gm/dl (median = 7.65). Platelet counts ranged from 10.00 to 160.00 x 109/L (median = 36.50). The percentage of peripheral blood blasts was between 20.00 and 96.00% (median = 61%). The blast percentage in the BM was between 22.00 and 92. 00% (median = 65.5%).

Immunophenotyping results showed that CD13, 33, and 34 were positive in 100% of cases, HLA-DR in 90% of cases, CD14 in 22.5% of cases, and lymphocyte markers in 100% of cases.

Cytogenetic and molecular analysis results are available for 21 patients, with t(8;21) detected in 14 (35%), 46xx del6 detected in 1, and t(15,17) detected in four cases. One patient had t(16,16), and one had normal 46xy.

Regarding FAB classification, 3/40 cases (7.5%) of the examined cases were diagnosed as M0, 8/40 cases (20%) as M1, and 14/40 cases (35%) as M2.4/40 cases (10%) were diagnosed as M3, 4/40 cases (10%) were diagnosed as M4, 5/40 cases (12.5%) were diagnosed as M5, and 1/40 cases

(2.5%) were diagnosed as M6, and 1/40 cases (2.5%) were diagnosed as M7.

The expression of miR-424s in both patients and controls is summarized in (Table 1).

Table 1: MiR- 424 in AML patients and controls

Variable		Patients (N=40)		Controls (N=40)		*P value*
		Count	%	Count	%	
miR-424	detectable	27	67.5%	40	100%	0.0001* (HS)
	undetectable	13	32.5%	0	0%	

Table 2: The real-time results of the studied genes expressed as CT

	Patients (no=40)	Controls (no=40)	*P value*
Mir -424	Median(range) 35.479 (0-42.53)	Median(range) 36.455 (35.42-38.35)	0.87(NS)
RNU6B	Median(range) 31.05 (24.67-41.17)	Median(range) 32.45 (28.11-34.49)	0.79(NS)

Regarding Mir-424 and RNU6B, there were no marked differences between the mean CT values of patients and controls (*p-value* = 0.87 and 0.79, respectively).

The level of each gene is $2^{-(\Delta\Delta CT)}$, i.e., as the number exceeded the mean concentration of controls. As mentioned earlier in the statistical section, patients were divided into two groups based on these values: an upregulated group and a downregulated group. The values of Mir-424 were 20.1750 (1.566 to 36.164) in the upregulated group and 0.2227 (0.0106 to 1.1987) in the downregulated group.

Table 3: Results of Mir -424 in patients group

	Mir -424	
	Upregulated	Downregulated
Median	20.1750	0.2227
Range	1.566- 36.164	0.0106- 1.1987

There were no statistically significant correlations between demographic or laboratory data and miR-424 in patients or controls (Tables 4,5).

Table 4: Correlation between demographic, laboratory data & miR- 424 among patients

parameter	miR-424	
	r	*p*
Age	-0.367	0.059
Initial Hb(g/dl)	-0.166	0.409
Intial TLC(x109/L)	-0.140	0.488
Initial platelets(x109/L)	-0.109	0.588
Intial blast%(PB)	-0.141	0.482
Intial blast%(BM)	-0.134	0.506
LDH(u/l)	-0.145	0.531

Table 5: Correlation between demographic, laboratory data & both miR-424 relations among controls

parameter	miR-424	
	r	*p*
Age	0.110	0.500
Initial Hb(g/dl)	0.019	0.907
Initial TLC(x109/L)	0.053	0.746
Initial platelets(x109/L)	-0.120	0.460

NPM1 polymorphism in AML Patients and controls

In the patients' group:

AA (wild type) was detected in 0/40 cases (0%), AG genotype (heterozygote) in 31/40 cases (77.5%), and GG genotype (homomutant) in 9/40 cases (77.5%).

In the control group:

AA (wild type) was detected in 5/40 (12.5%) control subjects, AG genotype (heterozygous) was detected in 31/40 (77. 5%) controls, whereas GG genotype (homomorphic variant) was detected in 4/40 controls (10%).

We compared the frequency of NPM1 genotypes among patients based on sex and found no statistically significant differences (P = 1.000). When examining the relationship between the age of onset of AML and the frequency of NPM1 genotype in patients, no substantial differences were found (P=0.358).

Regarding patient symptoms and signs, no statistically significant differences were found between NPM1 genotypes (p = 0.749 and 0.215, respectively). Hematological examination of the patients revealed no correlations between NPM1 genotypes that were statistically significant. In addition, no substantial differences were detected between NPM1 genotype and miR-424 (p = 0.426) (Table 6).

Table 6: Relation between NPM1 mutant genotypes among AML patients and miRNAs

Variable	Heterozygous (GG)		Homozygous mutant (AG)		*P value*
	Median	Range	Median	Range	
miR-424	3.07	0.02-10.65	1.00	0.03-9.49	0.426(NS)

Logistic regression analysis was performed to test whether there were significant predictors of AML. Age, sex, and NPM1 were included in the regression model.

Table 7: Prognostic predictors for AML among the studied group(follow up period after 18 months)

	Odds ratio (OR)	95.0% C.I.for (OR)	*P-Value*
Age	1.116	1.046 - 1.191	0.001 (HS)
Sex(f/m)	0.824	0.239 - 2.843	0.759 (NS)
G allele (AG+GG)	5.249	1.008 - 27.322	0.049 (S)

Gender differences have no prognostic significance; poor prognosis does not show a preference for a specific sex.

People over 60 years old and those with a mutation in the NPM gene (G allele) were identified as potent predictors of poor outcomes. Each factor independently contributes to a higher risk of developing AML.

DISCUSSION

The prevalence of hematopoietic and lymphoid cancers in Egypt rose by about 11-fold from 1972 to 2001[23]. In 2010, leukemia ranked as the fourth most prevalent cancer in Egypt, accounting for 7.2% of cases. In 2014, WHO statistics reported that Egypt had an age-standardized mortality rate of 5.55 per 100,000 inhabitants for leukemia, ranking 19th globally[24].

The precise cause of leukemia remains unidentified despite thorough research into its mechanisms.AML is a diverse disease where around 55% of patients show recurring cytogenetic changes, while the remaining 45% do not display any visible karyotypic lesions. The subgroup known as cytogenetically normal AML (CN-AML) is distinguished by subtle mutations that impact multiple genes. NPM1 alterations account for approximately 60% of CN-AML cases[25]. It is located on 5q35.1 and contains 12 exons. It is mainly found in the nucleolus and is believed to act as a molecular chaperone for proteins, promoting centrosome duplication in addition to facilitating the movement of ribosomal proteins through the nuclear membrane[26].

When NPM1 is in the cytoplasm, it cannot carry out its normal roles as a binding partner and transporter, indicating that mutations in NPM1 might be an early event in leukemogenesis[20]. NPM1 plays a significant role in leukemia and lymphoma as it is involved in the formation of fusion proteins resulting from chromosomal translocations.

Nearly 8% of cells in non-Hodgkin's lymphoma in children and young adults exhibit the t(2;5)(p23;q35) chromosomal translocation, leading to a chimeric fusion of NPM1 and ALK (anaplastic lymphoma kinase).The chromosomal translocation t(3;5)(q25.1;q34) is rarely detected.Myelodysplastic syndromes and AML result in symptoms like A fusion transcript involving NPM1 and MLF1[27].

MiRNAs are a new type of endogenous noncoding RNAs, typically 19 to 25 nucleotides in size, that regulate gene expression mainly after transcription and play a role in various cellular activities like apoptosis, differentiation, and cell proliferation.

Recent evidence indicates that abnormal expression of specific miRNAs acting as tumor suppressors or oncogenes is linked to the development of leukemia. Furthermore, distinct miRNA expression patterns can accurately categorize different types of human leukemias[28]. Circulating miRNAs have the potential to serve as cancer biomarkers due to their high stability capacity to denote tumor status and anticipate treatment response. They are characterized by tissue-specific expression, and their signatures are helpful for cancer classification. Their existence in the body enables analysis through less intrusive techniques, thus rendering biomarker analysis more feasible in clinical settings[29].

Furthermore, tumors release exosome miRNAs to enhance cell communication, protecting them from ribonuclease degradation within these vesicles. The upregulated miRNAs are mainly linked to increased cell cycle progression and reduced apoptosis, which are typical features of cancer cells[30].

MiR-424 is crucial in regulating various essential cellular processes, including differentiation, migration, and cell proliferation. MiR-424 was recently categorized into a large cluster family along with miR-15/miR-16. Its members are generally considered tumor suppressors since they accelerate apoptosis and hinder proliferation[4]. It specifically targets the primary transcript of mir-9, which is a microRNA that inhibits cell differentiation[31].

A quantitative reverse transcription-PCR (qRT-PCR) assay was used to measure the levels of miR-424 in this study. We aimed to determine the role of miRs in AML and establish a correlation between the expression levels of two miRs and NPM1 utilizing RT-PCR.

Consequently, RT-PCR was performed at the National Cancer Institute on 40 newly diagnosed AML adults, with a mean age of 41.63 ± 13.26 years and a male-to-female ratio of 0.9: 1. In addition, 40 healthy volunteers of matching age and sex served as controls. Their mean age was 32.45 ± 7.33 years, and the male: female ratio was 0.7: 1. Age is a significant independent prognostic factor in AML patients.

In our logistic regression analysis, we hypothesized that each additional year of age would correlate with an elevated risk of developing AML (Table 6). This finding aligns with the 2017 leukemia estimates from the American Cancer Society, indicating that AML is typically found in older individuals and becomes more common after age 45[32].

According to our study,there was highly statistically significant difference in miR- 424 between both patients and controls where *P value* =(0.0001). It was detectable in 27/40 (67.5%) of patients while in control group it was detectable in 40/40 cases(100%) which indicate that miR-424 may play a protective role in leukomogenesis.

This was in accordance to a study done by Faraoni *et al*,2012 who stated that miR-424 act as a tumor suppressor gene targeting the BCL2 oncogene, and can inhibit cell proliferation and promote apoptosis of leukemic cells[33].

MiR-424 was up-regulated in 15 cases and 4 controls with statistically significant difference *p value*= (0.04) and down regulated in 12 cases and 36 controls with statistically significant difference *p value*= (0.0035)

.Our results was in contrast to a study done by Yan *et al*.,2016[13] who discovered that over-expression of miR-424 may play a role in leukemogenesis by increasing sensitivity of AML cells to TRAIL (TNF-related apoptosis inducing ligand),a member of the TNF family expressed mainly by immune system,which is able to trigger extrinsic and intrinsic apoptosis that specifically kill tumor cells.

Kouchkovsky and Abdul-Hay, 2016 also noted that the frequency of AML rises with age. Despite current treatments, up to 70% of patients aged over 65 die within a year of diagnosis, with a bleak prognosis for older individuals who compromise the majority of new cases[34].

The study observed no marked differences in miR-424 expression level between AML patients and controls ($P = 0.87$), suggesting that miR-424 does not play a role in leukocyte formation. In contrast to Faraoni *et al*. 2012, this study found that miR-424 acts as a tumor suppressor gene by inhibiting cell proliferation and promoting apoptosis of leukemic cells associated with the BCL2 oncogene[16].

MiR-424 was elevated in 20 cases, decreased in 7 cases, and undetected in 13 cases. Yan *et al*., 2016 illustrated that miR-424 overexpression is implicated in leukemogenesis by elevating AML cell sensitivity to TRAIL (TNF-related apoptosis-inducing ligand), a member of the TNF family that is primarily expressed. This can induce both extrinsic and intrinsic apoptosis, leading to the targeted destruction of tumor cells. MiR-424 enhances TRAIL sensitivity in AML by post-transcriptionally regulating PLAG1, a recognized oncogenic gene. The mRNA levels of PLAG1 show an inverse relationship with miR-424 expression, causing an increase in miR-424 levels. Epigenetic silencing of PLAG1 occurs when miR-424 is downregulated, leading to an overexpression of PLAG1 and subsequently increasing TRAIL resistance[28].

The study revealed no statistically significant correlation between AML cases with miR-424 expression and NPM1 mutations. In contrast to Faroni *et al*. 2012, who found a significant decrease in miR-424 in CN-AML with NPM1 mutations, indicating that reduced miR-424 expression in NPM1 mutations plays a crucial role in AML development[25].

CONCLUSION

Finally, the above discussion suggests that miR-424 does not play a role in leukemogenesis in adult AML patients. Expression of a single miRNA can complement and extend current knowledge about AML.AML is relatively easy to measure to determine the molecular risk assessment of an individual patient at the time of diagnosis.We are just beginning to uncover the functional relationship between miRNAs and AML.

Therefore, this study reveals the diagnostic and prognostic value of miR-424, identifies its role in stratifying therapeutic strategies, and more importantly, identifies its potential as a future therapeutic agent in AML.

CONFLICT OF INTERESTS

There arfe no conflicts of interest.

Mir-424 is not involved in the development of her AML.

No correlation was found between miR-424 and NPM1.

REFERENCES

1. Grimwade D HB, Ivey A. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. Blood. 2016;127(1):29-41.
2. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, *et al*. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–47.
3. D Grimwade , H Walker, F Oliver, K Wheatley, C Harrison, G Harrison *et al*. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC The Medical Research Council Adult and Children's Leukaemia Working Parties.Blood1998 1;92(7):2322-33.
4. M L Slovak , K J Kopecky, P A Cassileth, D H Harrington, K S Theil, A Mohamed, *et al*. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology .Blood 2000 15;96(13):4075-83.
5. Joseph D. Khoury , Eric Solary , Oussama Abla , Yassmine Akkari , Rita Alaggio , Jane F. Apperley *et al*. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/ Dendritic Neoplasms. 2022
6. Stéphanie Nguyen , Thierry Leblanc, Pierre Fenaux, Francis Witz, Didier Blaise, Arnaud Pigneux, *et al*. A white blood cell index as the main prognostic factor in t(8;21) acute myeloid leukemia (AML): a survey of 161 cases from the French AML intergroup. Blood. 2002 15;99(10):3517-23.
7. Elias Campo , Steven H Swerdlow, Nancy L Harris, Stefano Pileri, Harald Stein, Elaine S Jaffe The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood . Blood. 2011 12;117(19):5019-32.
8. Deng Y, Cheng Q, He J. HDAC inhibitors: Promising agents for leukemia treatment Biochemical and Biophysical Research Communications.2023;680:61-72.

9. Xia Jiang ,Ye Lin ,, Mengting Zhao, Youhong Li , Peipei Ye , Renzhi Pei *et al*. Platycodin D induces apoptotic cell death through PI3K/AKT and MAPK/ERK pathways and synergizes with venetoclax in acute myeloid leukemia. Eur J Pharmacol 2023:956:175957

10. Chan Shan , Yan Liang , Kun Wang , Peifeng Li . Noncoding RNAs in cancer ferroptosis: From biology to clinical opportunity .Biomed Pharmacother. 2023 165:115053.

11. Jaffe E, Arber DA, Campo E, Harris NL, Quintanilla-Fend L. Hematopathology, 2nd ed. New York, NY: Elsevier; 2016.

12. Xiaolu Song , Ye Peng , Xiaogang Wang , Yirui Chen , Lai Jin, Tianxin Yang *et al*. Incidence, survival, and risk factors for adults with acute myeloid leukemia not otherwise specified and acute myeloid leukemia with recurrent genetic abnormalities: analysis of the surveillance, epidemiology, and end results (SEER) database, 2001–2013. Acta Haematol. 2018; 139(2): 115-127.

13. Yan-Ping Sun , Fei Lu , Xiao-Yu Han , Min Ji , Ying Zhou , A-Min Zhang *et al*,(2016): miR-424 and miR-27a increase TRAIL sensitivity of acute myeloid leukemia by targeting PLAG1 .Oncotarget; 7(18): 25276–25290.

14. George A Calin , Amelia Cimmino, Muller Fabbri, Manuela Ferracin, Sylwia E Wojcik, Masayoshi Shimizu *et al*: MiR-15a and miR-16-1 cluster functions in human leukemia. Proc Natl Acad Sci U S A . 2008;105(13):5166-71.

15. Aqeilan RI, Calin GA, Croce CM: miR-15a and miR-16-1 in cancer:discovery, function and future perspectives.Cell Death Differ. Cell Death Differ . 2010;17(2):215-20.

16. Silvia Grisendi , Cristina Mecucci, Brunangelo Falini, Pier Paolo Pandolfi Nucleophosmin and cancer. Nat Rev Cancer6.2006 (7):493-505.

17. B Falini, D Lenze, R Hasserjian, S Coupland, D Jaehne, C Soupir, Cytoplasmic mutated NPM defines the molecular status of a significant fraction of myeloid sarcomas. Leukemia2 ;2007; 1 (7): 1566-70.

18. B Falini , N Bolli, A Liso, M P Martelli, R Mannucci, S Pileri, I Nicoletti Altered nucleophosmin transport in AML with mutated NPM 1: molecular basis and clinical implications. Leuk23.2009; (10),1731-43.

19. N Bolli, M F De Marco, M P Martelli, B Bigerna, A Pucciarini, R Rossi *et al*. A dose dependant tug of war involving the NPM 1 leukemic mutant, nucleophosmin and ARF. Leukemia.2009 ; 23 (3), 501-9.

20. Barbora Brodská Markéta Kráčmarová , Aleš Holoubek, Kateřina Kuželová Localization of AML-related nucleophosmin mutant depends on its subtypeNand is highly affected by its interaction with wild-type NPM. PLoS ONE.2014 ; 12(4): :e0175175.

21. Mestdagh P, Van Vlierberghe P, De Weer A, Muth D, Westermann F, Speleman F *et al*. A novel and universal method for microRNA RT-qPCR data normalization. Genome biology.2009 ; 10(6):R64

22. Schmittgen TD, Livak KJ . Analyzing real-time PCR data by the comparative C T method. Nature protocols.2008 3(6):1101.

23. Hosny G and Elkaffas SM Patterns in the incidence of pediatric cancer in Alexandria, Egypt, from 1972 to 2001. J.Egypt Public Health Assoc.2002 ; 77: 451-468.

24. National Cancer Institute (NCI) registry. National Cancer Institute Statistics Department of Biostatistics and Cancer Epidemiology, NCI Cairo.2010

25. Isabella Faraoni , Serena Laterza, Davide Ardiri, Claudia Ciardi, Francesco Fazi, Francesco Lo-Coco .miR-424 and miR-155 deregulated expression in cytogenetically normal acute myeloid leukaemia:correlation with NPM1 and FLT3 mutation status.Journal of Hematology & Oncology.2012 ; 5(26):1-5.

26. Jingrong Xian, Huiyuan Shao , Xianchun Chen, Shuaishuai Zhang , Jing Quan , Qin Zou *et al*. Nucleophosmin Mutants Promote Adhesion, Migration and Invasion of Human Leukemia THP-1 Cells Through MMPs Up-Regulation via Ras/Erk MAPK Signaling. Int J Biol Sci.2016 12 (2), 144-155.

27. Roel G W Verhaak, Chantal S Goudswaard, Wim van Putten, Maarten A Bijl, Mathijs A Sanders, Wendy Hugens *et al*. Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance.Blood.2005; 106:3747-3754.

28. Chen CZ (2005):MicroRNAs as oncogenes and tumor suppressors. N Engl J Med 2005;353:1768-71.

29. Yasuto Kinose, Kenjiro Sawada, Koji Nakamura , Tadashi Kimura . The Role of MicroRNAs in Ovarian Cancer. BioMed Research International.2014; 1-11.

30. Calin G A, Amelia Cimmino , Muller Fabbri : MiR-15a and miR-16-1 cluster functions in human leukemia. BiolReprod.2008 ; 105(3): 5166–5171.

31. Forrest A, Kanamori-Katayama M, Tomaru Y *et al*., (2010) Induction of microRNAs, mir-155, mir-222, mir-424 and mir-503, promotes monocytic differentiation through combinatorial regulation. Leukemia (2010) 24, 460–466.

32. American Cancer Society: Cancer Facts & Figures 2017:1-76.

33. Faraoni I, Laterza S, Ardiri D, *et al*., (2012): miR-424 and miR-155 deregulated expression in cytogenetically normal acute myeloid leukaemia:correlation with NPM1 and FLT3 mutation status.Journal of Hematology & Oncology; 5(26):1-5.

34. De Kouchkovsky and M Abdul-Hay: Acute myeloid leukemia: a comprehensive review and 2016 update. Blood cancer journal 2016; 6(7): e441.