Iraqi Journal of Biotechnology, 2026, Vol. 25, No. 2, 83-95

Gene expression of *CEBPA* in Iraqi patients with acute myeloid leukemia

[1]Noor Hussein Ali, [1]Nuha J. Kandala

[1]Department of Biotechnology, College of Sciences, University of Baghdad.

Abstract

Background. One of the most common genetic abnormalities in acute myeloid leukemia (AML) is mutations in the CCAAT enhancer binding protein alpha (*CEBPA*) gene. This gene codes for crucial transcription factors for differentiation and controlling the proliferation of myeloid precursors. **Aim.** To analyze the relative mRNA expression of *CEBPA* in AML patients and its correlation with clinical, morphological, cytogenetic, and gene mutation parameters among Iraqi patients for the first time. **Methods.** Utilizing quantitative real-time polymerase chain reaction (RT-qPCR), the expression of the *CEBPA* gene was examined in the peripheral blood of 120 patients and 40 controls. The AML patients were characterized in terms of age, sex, FMS-like tyrosine kinase 3 internal tandem duplication (*FLT3-ITD*) and Nucleophosmin1 (*NPM1*) mutations, the French American British classification (FAB), and the World Health Organization (WHO). **Results**. The findings revealed that the expression levels of *CEBPA* were lower in patients compared to healthy controls, indicating gene downregulation. The expression was lower in females than males (p = 0.025) and decreased across disease stages. In addition, the FAB M1 type and WHO RUNX1T1/RUNX1 had the lowest *CEBPA* mRNA expression among the other types. The mutations of *FLT3-ITD* and *NPM1* significantly affected *CEBPA* expression, with *FLT3-ITD* having the most pronounced effect (p = 0.029). **Conclusion**. The mRNA expression of the *CEBPA* gene was downregulated in patients with AML, especially in females and those with the FAB M1 type and the WHO RUNX1T1/RUNX1. Additionally, both *FLT3* and *NPM1* genes significantly influenced *CEBPA* expression.

Keywords: *CEBPA* expression, RT-qPCR, AML, Iraq.

Corresponding author: (Noor.ali@sc.uobaghdad.edu.iq)

Introduction

Acute myeloid leukemia (AML) is a heterogeneous group of diseases with varying prognoses, courses of the disease, and therapeutic responses. The risk categorization of AML has been improved with the incorporation of cytogenetic data in the classification process (1). A combination of genetic and environmental factors, including Down syndrome, Fanconi anemia, and exposure to radiation, alkylating drugs, or topoisomerase II, may contribute to the development of most cases of AML (2). Acquired genetic and clonal chromosomal abnormalities are present in 50–80% of patients with AML, particularly in older individuals and those with secondary leukemia (3). These abnormalities include losing or deleting chromosomes 5, 7, Y, and 9. Additionally,chromosome translocations are common, including t(8;21) (q22;q22), t(15;17) (q22;q11), as well as trisomy of chromosomes 8 and 21. Abnormalities are also observed in chromosomes 16, 9, and 11(4, 5).

Numerous mutations have been identified in AML patients, most commonly involving the FMS-like

tyrosine kinase 3-internal tandem duplication (*FLT3-ITD*) and Nucleophosmin 1 (*NPM1*) genes. These mutations significantly impact both diagnosis and treatment (6). *NPM1* mutations are generally linked to more favorable outcomes, particularly when *FLT3* mutations are absent. Additionally, there is often a positive relationship between *CEBPA* and *NPM1* (7).

In addition to genetic heterogeneity, the French-American-British (FAB) Collaborative Group system classifies AML into eight subtypes (FAB M0 to M7) based on the type of cell from which the leukemia originates and the maturity level of those cells (8). This classification is important because AML exhibits a wide variety of morphological and cytochemical characteristics (9).

The most common genetic abnormalities in AML are mutations in the *CEBPA* gene, which are found in approximately 10% to 15% of AML cases (10).

The *CEBPA* gene is a single-exon gene located on chromosome 19q13.1, coding for 358 amino acids (11). The CEBPA protein localizes to the nucleus and functions as a transcription factor. It has four principal domains: a basic leucine zipper (bZIP) domain, which consists of a basic region that interacts with specific DNA sequences (DNA-binding domain), and a leucine zipper domain that facilitates homo- or heterodimerization at the COOH terminus. Additionally, it contains two transactivation domains, TAD1 and TAD2, located at the NH2 terminus (12, 13).

The *CEBPA* mRNA can be translated into two isoforms: the p42 protein and the p30 protein (14). The p42 protein belongs to the CCAAT/enhancer binding protein family and plays a crucial role in balancing cell proliferation and terminal differentiation. In contrast, the p30 protein lacks the normal functions of CEBPA due to the absence of the TAD1 functional domain. As a result, it exerts a dominant negative effect on the wild-type p42 protein (15).

This study aimed to analyze the relative mRNA expression of *CEBPA* in patients with AML and its correlation with age, sex, treatment, and *FLT3-ITD* and *NPM1* mutations. In addition to the evaluation of FAB and WHO classifications.

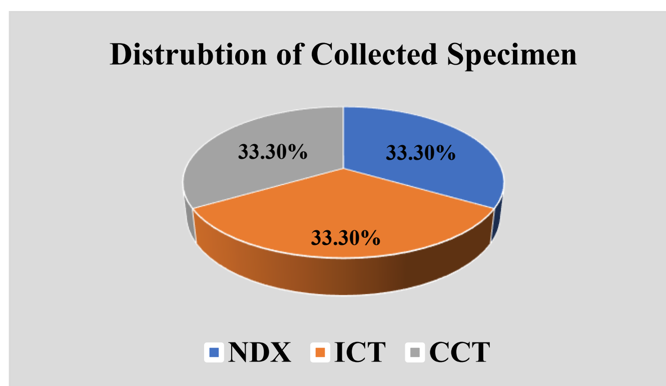

Figure (1): Distribution of the collected samples depending on the AML status, NDX= 40 newly diagnosed, ICT= 40 induction chemotherapy, and CCT= 40 consolidation chemotherapy.

Materials and Methods

Ethical Approval

The University of Baghdad, College of Science, Ethics Committee granted ethical permission for this study, with a reference number of CSEC/1123/0070.

Specimen collection

At the National Center for Hematology in Baghdad, Iraq, 120 blood samples were gathered from patients with AML whose ages ranged 14 - 80 years old and were divided according to sex into 60 males and 60 females. Among these, 40 were newly diagnosed (NDX), 40 had induction chemotherapy (ICT), and 40 patients were in the consolidation chemotherapy stage (CCT), as shown in Figure 1. Participants were enrolled from September 20, 2023 to May 10, 2024. AML was diagnosed and classified according to FAB (M0-M7) and WHO (PML/RARE, RUNX1T1/RUNX1, and MYH11/CBFB) guidelines, which included clinical, morphological, biochemical, cytogenetic, and gene mutation assessments. Furthermore, age, sex, WBC count, platelet count, disease status, FLT-ITD and NPM1 mutations were reported from the case history. Patients with diabetes, infectious diseases, Down syndrome, and other chronic diseases were excluded. Moreover, 40 controls aged between 14 and 80 years were divided according to sex into 20 males and 20 females. Blood samples were acquired from the National Blood Bank in Baghdad, Iraq.

Total RNA Extraction

Using TRIzol™ (Thermo Scientific, UK), the RNA was separated from the blood specimens. An aliquot of 0.25 ml of blood from both patients and controls was added to 0.75 ml of TRIzol. The instructions of the manufacturer were followed.

Estimation of RNA purity

A Nanodrop spectrophotometer 2000c (Thermo Fisher Scientific, USA) was used to determine the concentration and purity of the extracted RNA.

Synthesis of the cDNA from whole RNA

To generate complementary DNA (cDNA), the EasyScript® First-Strand cDNA Synthesis SuperMix Kit (Transgene, China) was utilized. The whole procedure was performed as per the instructions provided by the manufacturer. The conditions for the RNA to cDNA synthesis are shown in Table (1).

Table (1). Conditions for the RNA to cDNA synthesis using thermal cycler steps.

	STEP 1	STEP 2	STEP3
Temp.	25 ºC	42 ºC	85 ºC
Time	10 min	15 min	5 sec

Primer Design

The cDNA sequences of the *CEBPA* and *GAPDH* genes were obtained from the NCBI GenBank database. RT-qPCR primers were designed in this study using Premier 3 software. Table (2) shows the primers' details.

Table (2). The sequences and details of the primers used in this study.

		Primer Sequences 5′→3′	GC %	Tm (˚C)	Product Size (pb)
GAPDH	**F**	GTCTCCTCTGACTTCAA	47	50	131
GAPDH	**R**	ACCACCCTGTTGCTGTA	53		
CEBPA	**F**	CATGAGAGAAGGAGGGGAGCA	57	60	197
CEBPA	**R**	AGAGGCGTGGAACTAGAGACC	57		

Primer preparation

The CEBPA and GAPDH primers, manufactured by Alpha DNA Ltd. (Canada), were lyophilized and dissolved in nuclease-free water as specified by the manufacturer's instructions. A stock solution of 100 pmol/µl was prepared for each primer and stored at -23°C. 10 µl of the stock solution of the primers was diluted with 90 µl of nuclease-free water and kept at -20˚C until needed.

RT-qPCR protocol

This protocol involved cDNA samples from both the patient and the control within one run. For each sample, two PCR tubes were utilized: one for studying the experiment of *CEBPA* and the other for *GAPDH*, which was used as a housekeeping gene in this study. The CyberGreen fluorescence method was applied (Thermo Fisher Scientific, USA) and was used in the current investigation. The reaction mix was prepared using the Luna Universal qPCR Master Mix (New England Biolabs, USA). The components of the reaction, along with their respective quantities are listed in Table (3)

Table (3). Components used for the RT-qPCR.

Component	Concentration	Volume
Luna Universal qPCR Master Mix	2x	10µl
F primer	10µM	1µl
R primer	10µM	1µl
cDNA	25-27 ng/µl	4µl
Nuclease-free water	-	4µl
Total volume	**-**	**20µl**

Table (4). Gene expression thermal profile.

Cycle step	Temp.	Time	Cycles
Initial Denaturation	95ºC	60 sec	1
Denaturation **Annealing** **Extension**	95ºC 60ºC 72ºC	15 sec 30 sec 30 sec	45
Melt curve	60-95ºC	40 min	1

Using the real-time cycler software, the threshold cycle (CT) was calculated for each sample. All samples were analyzed in duplicate, and mean values were determined. The expression data of the target gene were normalized against the housekeeping gene. The ΔΔCt method of Livak and Schmittgen (16) was used for data analysis, and the results were expressed as a fold change in gene expression. For each sample, the difference in CT values (ΔCt) between the target gene and the housekeeping gene was calculated [ΔCt healthy or patient =CT target gene-CT housekeeping gene]. The differences in ΔCt values (ΔΔCt) for the gene of interest were calculated as follows: ΔΔCt=ΔCt(patient)-ΔCt (healthy). The fold-change in gene expression was calculated as follows:

Fold change = $2^{-\Delta\Delta Ct}$

Statistical Analysis

The statistical software IBM SPSS version 25.0 (Armonk, NY: IBM Corp) was utilized. The Mann-Whitney U test was used for comparisons between two the groups, and the Kruskal-Walli's test for comparisons involving more than two groups. A probability value (p) of less than 0.05 was considered statistically significant. The p-values were corrected using Bonferroni

correction for multiple comparisons (17).

Results

Quantitative Expression of the *CEBPA* gene

The whole cell RNA was successfully extracted from all samples. The RNA had a high purity with a ratio >1.8 at an OD 260/280 nm. The RNA concentration in the samples ranged between 73 and 147 ng/µl. Quantitative PCR (qPCR) was employed to determine the level of cDNA in the studied samples. The findings in Figures (2) and (3) show the amplification and melting plots of the *CEBPA* and *GAPDH* genes of the patients and healthy subjects using RT-qPCR.

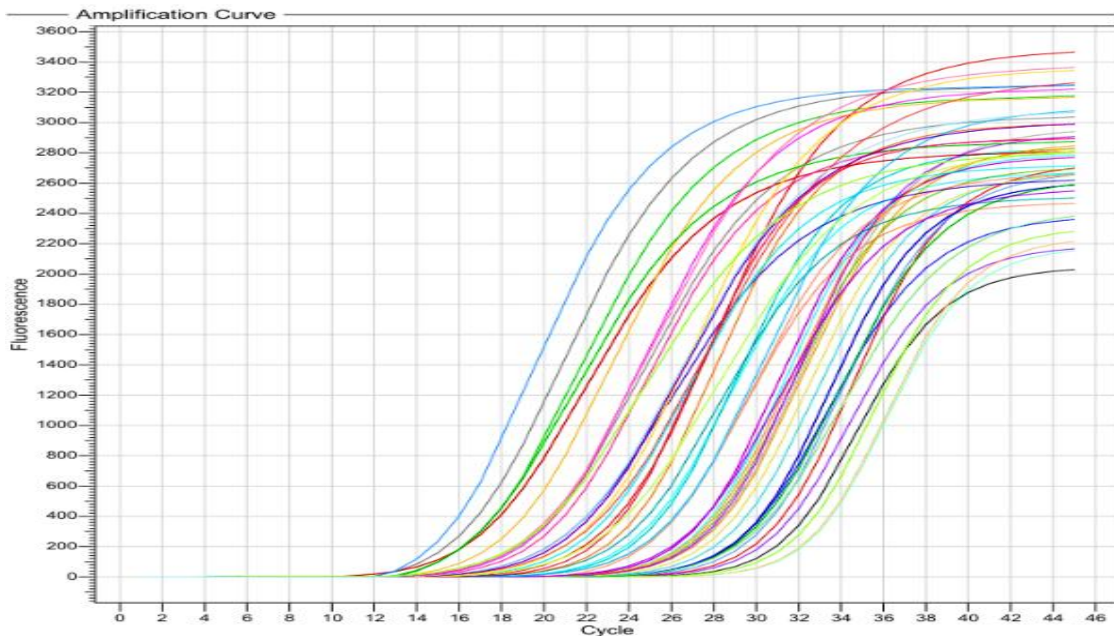

Figure (2). The amplification plots of the target *CEBPA* and *GAPDH* genes of the patients and control subjects using RT-qPCR. Each curve represents a separate specimen.

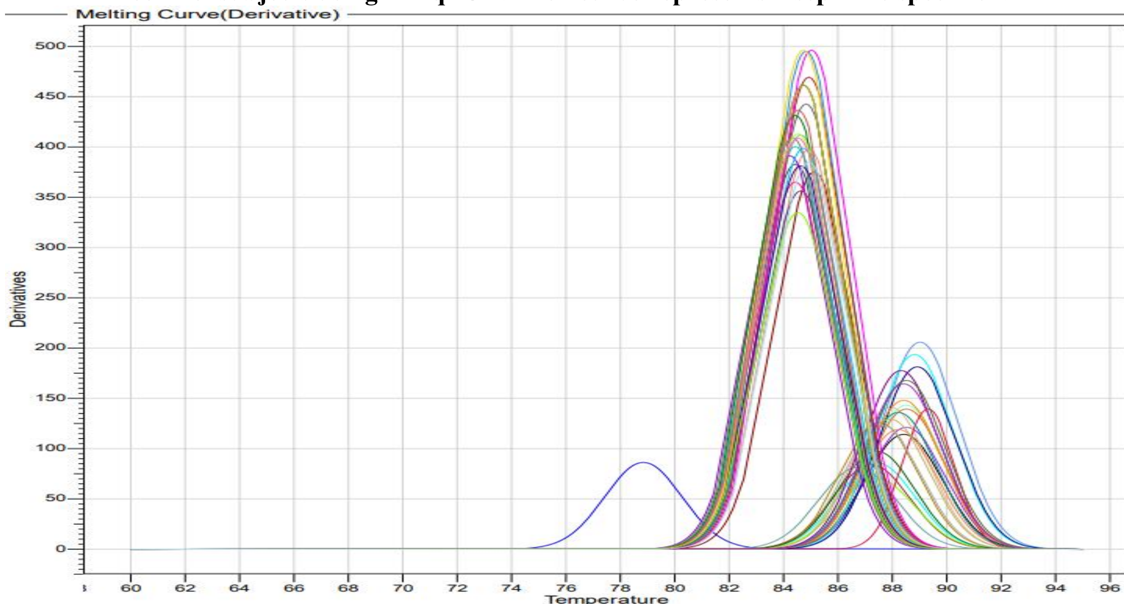

Figure (3). Melting curves of the *CEBPA* and *GAPDH* genes of different subjects of the patients and control after RT-qPCR analysis.

The expression level of the *CEBPA* gene in the studied groups

The results revealed that AML patients exhibited a reduced level of *CEBPA* mRNA expression, with a relative expression value of 0.48 compared to the control group (Figure 4). This finding indicates a decrease in *CEBPA* gene expression among patients with AML.

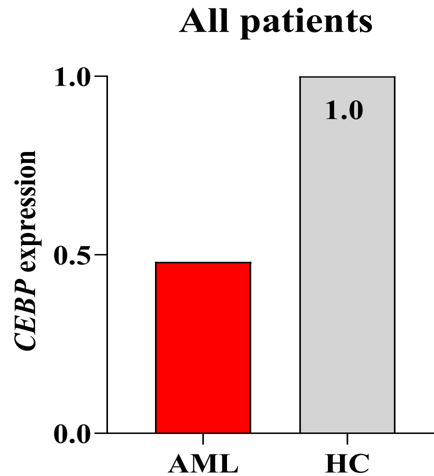

Figure (4). Fold change in the *CEBPA* gene expression in patients with AML compared to healthy controls (HC). Expression was given as a median and interquartile range (IQR) of 0.48 (0.26-1.32).

Nonetheless, there might be a distinction between AML patients and controls in the connection between sex and *CEBPA* expression. The downregulation in expression was more noticeable in females (0.35) than in males (0.60), with a significant difference (p = 0.025). Similar results were also obtained by (21) as shown in Table (5).

Table (5). Fold change in the *CEBPA* gene expression between patients according to sex

Sex	*CEBPA* expression (Fold change)		*p*-value
	Median	IQR 25–75 %	
Male	0.60	0.34-1.75	0.025*
Female	0.35	0.20-0.91	

*p-value indicates a significant value.

When AML patients were classified according to disease status into newly diagnosed (NDX), induction chemotherapy (ICT), and consolidation chemotherapy (CCT) groups, the relative expression levels of *CEBPA* mRNA showed a gradual decrease across the groups. The expression values were 0.56 in the NDX group, 0.54 in the ICT group, and 0.41 in the CCT group (Figure 5).

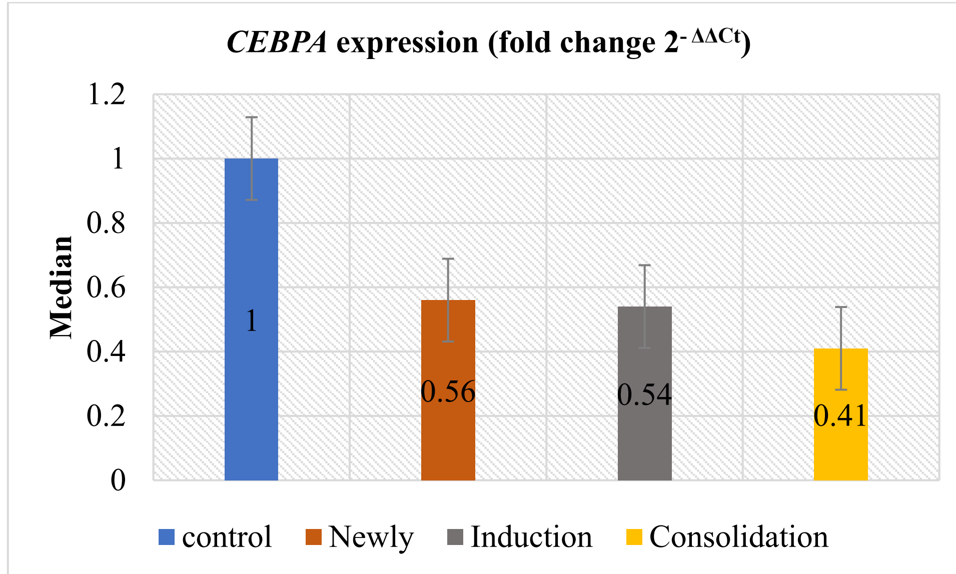

Figure (5): Fold change in the *CEBPA* gene expression in the control and patients according to the AML status.

As shown in Table (6), no significant association was observed between *CEBPA* gene expression and age in AML patients (p = 0.672). The relative expression level was 0.56 in patients younger than 40 years, 0.50 in those aged 40–50 years, and 0.39 in patients older than 50 years.

Similarly, no significant correlation was found between *CEBPA* expression and white blood cell (WBC) count (p = 0.546). Patients with WBC counts of <4.5 × 10^9/L and >12.0 × 10^9/L showed expression levels of 0.48, whereas those with WBC counts between 4.5 and 12.0 × 10^9/L exhibited a slightly higher expression level of 0.49.

Regarding platelet count, patients with platelet counts below 150 × 10^9/L had a higher *CEBPA* expression level (0.52) than those with platelet counts above 150 × 10^9/L, who showed an expression level of 0.42.

The expression of *CEBPA* mRNA varied among the FAB subtypes of AML. The lowest expression level was observed in the FAB M1 subtype (0.26), whereas the highest expression level was detected in the FAB M0 subtype (1.04) compared with the other AML subtypes. According to the WHO classification, AML patients with the *RUNX1T1*/*RUNX1* subtype exhibited low *CEBPA* expression (0.30), whereas patients with the *CBFB*/*MYH11* subtype showed the highest expression level (2.39).

Regarding molecular mutations, patients carrying *FLT3-ITD* mutations had significantly lower *CEBPA* expression (0.30) than those without the mutation (0.52) (P = 0.029). Similarly, patients with NPM1-positive mutations showed significantly lower *CEBPA* expression (0.39) than *NPM1*-negative patients (0.56) (P = 0.023).

Table (6). The *CEBPA* expression (measured by fold change) was classified based on the clinical characteristics of patients with AML.

Characteristic	Subgroup	Patients no. (N) =120	*CEBPA* expression (Fold change)		p-value
			Median	IQR 25–75 %	
Patients	All	120	0.48	0.26- 1.32	
Age group, year	<40	37	0.56	0.28-1.81	0.672
	40-50	56	0.50	0.21-1.32	
	>50	27	0.39	0.26-0.84	
WBC count (× 10^9/L)	< 4.5	51	0.48	0.28-1.69	0.546
	4.5-12.0	20	0.49	0.20-1.28	
	> 12.0	49	0.48	0.24-.97	
Platelets count (× 10^9/L)	< 150	102	0.52	0.24-1.28	0.752
	> 150	18	0.42	0.28-1.57	
Disease status	Newly	40	0.56	0.27-2.47	0.073
	Induction	40	0.54	0.29-1.15	
	Consolidation	40	0.41	0.19-0.76	
FAB classification	M0	6	1.04	0.20-1.28	0.643
	M1	1	0.26	0.26-0.26	
	M3	20	0.44	0.17-0.58	
	M4	7	0.60	0.28-0.79	
	M5	2	0.68	0.52-0.84	
	M7	2	0.29	0.28-0.30	
	UC	82	0.48	0.26-1.81	
FLT3-ITD	Positive	29	0.30	0.18-0.73	**0.029***
	Negative	91	0.52	0.28-1.81	
NPM1	Positive	42	0.39	0.23-0.90	**0.023***
	Negative	78	0.56	0.26-2.08	
PML/RARE	Positive	12	0.45	0.26-1.38	0.766
	Negative	108	0.50	0.26-1.32	
RUNX1T1/RUNX1	Positive	3	0.30	0.12-2.39	0.687
	Negative	117	0.48	0.26-1.28	
MYH11/CBFB	Positive	1	2.39	2.39-2.39	0.225
	Negative	119	0.48	0.26-1.28	

UC: unclassified, IQR: interquartile range; p: the two-tailed probability, *: indicates a significant value.

Discussion

The observed reduction in *CEBPA* expression is consistent with previous findings reported by Van Doorn et al. (18), who identified *CEBPA* down-regulation as a potential genetic biomarker for AML diagnosis. Several studies (19,20) have also demonstrated that decreased *CEBPA* expression

impairs the differentiation of myeloid progenitor cells into mature blood cells, thereby contributing to leukemogenesis. This down-regulation may arise from genetic alterations, including mutations in the N-terminal and C-terminal regions of the *CEBPA* gene, or from epigenetic mechanisms such as promoter hypermethylation and overexpression of microRNAs, particularly miR-124.The progressive reduction in *CEBPA* expression during treatment may be attributed to the effects of chemotherapy, which eliminates leukemic cells and consequently reduces the number of malignant cells expressing the gene (22). In addition, the replacement of leukemic cells with normal hematopoietic cells, which exhibit different gene expression profiles, together with chemotherapy-induced epigenetic modifications, may contribute to the observed decrease in *CEBPA* expression during the course of treatment (23). The absence of a significant association between *CEBPA* expression and age suggests that *CEBPA* dysregulation is more likely related to disease-specific molecular events, such as somatic mutations, rather than the aging process itself (24). Previous studies have reported that *CEBPA* mutations occur across different age groups, particularly in AML patients with normal karyotypes, without showing age dependency (25). Likewise, the lack of a significant relationship between *CEBPA* expression and WBC count is consistent with previous reports (26,27), indicating that *CEBPA* primarily regulates myeloid cell differentiation rather than cell proliferation. Loss or mutation of *CEBPA* impairs normal differentiation, contributing to leukemogenesis, whereas WBC count reflects disease burden rather than *CEBPA* expression alone. Although patients with lower platelet counts exhibited relatively higher *CEBPA* expression, this difference was not statistically significant. Platelet count in AML is generally associated with bone marrow infiltration by leukemic blasts and disease severity rather than direct regulation by *CEBPA*. Previous studies have shown that *CEBPA* mutations primarily affect myeloid differentiation and have limited influence on platelet production or platelet count in AML patients (28). The variation in *CEBPA* expression among FAB subtypes highlights the relationship between *CEBPA* and the degree of myeloid cell maturation. The relatively high expression observed in the FAB M0 subtype, despite impaired cellular differentiation, suggests that *CEBPA* may be expressed but functionally ineffective. In contrast, the markedly reduced expression in the FAB M1 subtype may reflect a more profound loss of *CEBPA* function, contributing to impaired differentiation and leukemogenesis (29). These findings support the potential value of *CEBPA* as a biomarker for distinguishing AML subtypes (30). The low expression of *CEBPA* in AML patients with the *RUNX1T1*/*RUNX1* subtype is consistent with the biological role of *RUNX1* in regulating *CEBPA* transcription. Dysfunction of *RUNX1* reduces *CEBPA* activation, resulting in impaired myeloid differentiation and promoting AML development. Reduced *CEBPA*

expression in this subtype may also be associated with more aggressive disease behavior and poorer therapeutic response (31). In contrast, the increased expression observed in the *CBFB*/*MYH11* subtype may represent a compensatory response to overcome the differentiation block induced by the fusion gene, although this increase appears insufficient to restore normal hematopoietic maturation (32). The significantly lower *CEBPA* expression in patients harboring *FLT3*-ITD mutations suggests that constitutive activation of signaling pathways, including STAT5 and MAPK, suppresses *CEBPA* function by promoting leukemic cell proliferation while inhibiting differentiation (33). Likewise, *NPM1* mutations were associated with reduced *CEBPA* expression; however, their effect is likely indirect through disruption of nucleolar function and altered regulation of transcription factors involved in *CEBPA* expression (34).

Conclusion

The mRNA expression of the *CEBPA* gene was downregulated in patients with AML compared to control subjects. These findings suggest that decreased expression of this gene may serve as a useful diagnostic marker and could potentially be targeted for treatment in AML cases in Iraq. When AML patients were categorized into three groups based on the disease status (NDX, ICT, and CCT), a gradual decline in *CEBPA* mRNA expression was observed. Notably, females, as well as those classified with the FAB M1 subtype and the WHO RUNX1T1/RUNX1 subtype, exhibited low levels of the *CEBPA* mRNA expression. Additionally, both *FLT3* and *NPM1* genes were found to significantly influence *CEBPA* expression.

References

1. Sahlol, A. T., Kollmannsberger, P., & Ewees, A. A. (2020). Efficient classification of white blood cell leukemia with improved swarm optimization of deep features. Scientific reports, 10(1), 2536.
2. Alyaqubi, K. J., AL-kaabi, A. J., & AL-kaabi, S. J. (2016). Plasma IL-10 Concentration and its role in the pathogenesis of acute myeloid Leukemia: A prospective study. Iraqi Journal of Biotechnology, 15(1).
3. AL-Emamein, A. L. (2023). The Role of Long Non Coding RNA ANRIL Gene Expression and Serum Interleukin-27 Level in Metastasis of Breast Cancer Patients. Iraqi Journal of Biotechnology, 22(1), 140-147.
4. Lafta, M., AL-Amili, W. A., Alwan, A. F., & Khalaf, I. A. H. (2022). Relationship between the drug responsiveness of acute myeloid leukemia Iraqi patients and gene expression of drug resistance ABCB1 and ABCG2 genes. Iraqi Journal of Biotechnology, 21(1).
5. D'Altri, T., Wilhelmson, A. S., Schuster, M. B., Wenzel, A., Kalvisa, A., Pundhir, S., Meldgaard Hansen, A., & Porse, B. T. (2021). The ASXL1-G643W variant accelerates the development of CEBPA mutant acute myeloid leukemia. Haematologica, 106(4), 1000–1007.
6. Juliusson, G., Jädersten, M., Deneberg, S., Lehmann, S., Möllgård, L., Wennström, L., ... & Höglund, M. (2020). The prognostic impact of FLT3-ITD and NPM1 mutation in adult AML is age-dependent in the population-basedsetting. Blood advances, 4(6), 1094-1101.
7. Zhao, Y., Huang, Y., Jiang, L., Zhang, Y., Liu, F., Yan, P., ... & Jiang, X. (2024). Impact of different CEBPA mutations on therapeutic outcome in acute myeloid leukemia. Annals of Hematology, 103(9), 3595-3604.
8. Canaani, J., Beohou, E., Labopin, M., Socie, G., Huynh, A., Volin, L., ... & Nagler, A. (2017). Impact of FAB classification on predicting outcome in acute myeloid leukemia, not otherwise specified, patients undergoing allogeneic stem cell transplantation in CR 1: An analysis of 1690 patients from the acute leukemia working party of EBMT. American journal of

hematology, 92(4), 344-350.
9. Jha, K. K., Das, P., & Dutta, H. S. (2020, July). FAB classification based leukemia identification and prediction using machine learning. In 2020 international conference on system, computation, automation and networking (ICSCAN) (pp. 1-6). IEEE.
10. Kantarjian, H., Kadia, T., DiNardo, C., Daver, N., Borthakur, G., Jabbour, E., ... & Ravandi, F. (2021). Acute myeloid leukemia: current progress and future directions. Blood cancer journal, 11(2), 41.
11. Boada, M., Catalán, A. I., Ottati, C., Bentancour, F., Lens, D., Guillermo, C., & Grille, S. (2021). Germline CEBPA mutation in familial acute myeloid leukemia. Hematology Reports, 13(3), 9114.
12. House, J. S., Gray, S., Owen, J. R., Jima, D. D., Smart, R. C., & Hall, J. R. (2023). C/EBPβ deficiency enhances the keratinocyte innate immune response to direct activators of cytosolic pattern recognition receptors. Innate Immunity, 29(1-2), 14-24.
13. Wakita, S., Sakaguchi, M., Oh, I., Kako, S., Toya, T., Najima, Y., ... & Yamaguchi, H. (2022). Prognostic impact of CEBPA bZIP domain mutation in acute myeloid leukemia. Blood Advances, 6(1), 238-247.
14. Heyes, E., Schmidt, L., Manhart, G., Eder, T., Proietti, L., & Grebien, F. (2021). Identification of gene targets of mutant C/EBPα reveals a critical role for MSI2 in CEBPA-mutated AML. Leukemia, 35(9), 2526-2538.
15. Nguyễn, T. L. (2022). Post-translational Modifications Of C/EBP Alpha p30 Regulate Its Functions In Leukemogenesis and Differentiation.
16. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. methods, 25(4), 402-408.
17. Pagano, M., Gauvreau, K., & Mattie, H. (2022). Principles of biostatistics. Chapman and Hall/CRC.
18. Van Doorn, S. B. V. W., Erpelinck, C., Meijer, J., van Oosterhoud, S., Van Putten, W. L., Valk, P. J., ... & Delwel, R. (2003). Biallelic mutations in the CEBPA gene and low CEBPA expression levels as prognostic markers in intermediate-risk AML. Hematol J, 4(1), 31-40.
19. Kassem, N., Fahmy, A. E. G., Desoky, M., Medhat, N., & Zawam, H. M. (2013). CCAAT/enhancer binding protein α gene expression in Egyptian patients with acute myeloid leukemia. Journal of the Egyptian National Cancer Institute, 25(3), 115-120.
20. Pulikkan, J. A., Tenen, D. G., & Behre, G. (2017). C/EBPα deregulation as a paradigm for leukemogenesis. Leukemia, 31(11), 2279-2285.
21. Gholami, M., Bayat, S., Manoochehrabadi, S., Pashaiefar, H., Omrani, M. D., Jalaeikhoo, H., ... & Mirfakhraie, R. (2019). Investigation of CEBPA and CEBPA-AS genes expression in acute myeloid leukemia. Reports of Biochemistry & Molecular Biology, 7(2), 136.
22. Hassan, N. M., Said, F., Shafik, R. E., & Abdellateif, M. S. (2021). Dysregulation of CCAAT/enhancer binding protein-alpha (CEBPA) expression in the bone marrow of acute myeloid leukemia patients. Egyptian Journal of Medical Human Genetics, 22, 1-10.
23. Döhner, H., Estey, E., Grimwade, D., Amadori, S., Appelbaum, F. R., Büchner, T., ... & Bloomfield, C. D. (2017). Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood, The Journal of the American Society of Hematology, 129(4), 424-447.
24. Tarlock, K., Lamble, A. J., Wang, Y. C., Gerbing, R. B., Ries, R. E., Loken, M. R., ... & Meshinchi, S. (2021). CEBPA-bZip mutations are associated with favorable prognosis in de novo AML: a report from the Children's Oncology Group. Blood, The Journal of the American Society of Hematology, 138(13), 1137-1147.
25. Medinger, M., & Passweg, J. R. (2017). Acute myeloid leukaemia genomics. British journal of haematology, 179(4), 530-542.
26. Merdin, A., Dal, M. S., Çakar, M. K., Yildiz, J., Ulu, B. U., Batgi, H., ... & Altuntaş, F. (2020). Analysis of pre-chemotherapy WBC, PLT, monocyte, hemoglobin, and MPV levels in acute myeloid leukemia patients with WT1, FLT3, or NPM gene mutations. Medicine, 99(14), e19627.
27. Taube, F., Georgi, J. A., Kramer, M., Stasik, S., Middeke, J. M., Röllig, C., ... & Study Alliance Leukemia (SAL). (2022). CEBPA mutations in 4708 patients with acute myeloid leukemia: differential impact of bZIP and TAD mutations on outcome. Blood, The Journal of the American Society of Hematology, 139(1), 87-103.
28. Shallis, R. M., Wang, R., Davidoff, A., Ma,

X., & Zeidan, A. M. (2019). Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. Blood reviews, 36, 70-87.

29. Pei, H., Peng, Q., Guo, S., Gu, Y., Sun, T., Xu, D., ... & Zhu, Z. (2020). MiR-367 alleviates inflammatory injury of microglia by promoting M2 polarization via targeting CEBPA. In Vitro Cellular & Developmental Biology-Animal, 56, 878-887.
30. Weinberg, O. K., Porwit, A., Orazi, A., Hasserjian, R. P., Foucar, K., Duncavage, E. J., & Arber, D. A. (2023). The International Consensus Classification of acute myeloid leukemia. Virchows Archiv, 482(1), 27-37.
31. Sendker, S., Schneider, M., Awada, A., Orhan, E., Sendker, M., Hoffmeister, L., ... & Von Neuhoff, N. (2022). RUNX1 Mutations Have Distinct Prognostic and Clinical Implication in Pediatric AML: Results of the AML-BFM Study Group. Blood, 140(Supplement 1), 9110-9112.
32. Opatz, S., Bamopoulos, S. A., Metzeler, K. H., Herold, T., Ksienzyk, B., Bräundl, K., ... & Greif, P. A. (2020). The clinical mutatome of core binding factor leukemia. Leukemia, 34(6), 1553-1562.
33. Su, L., Shi, Y. Y., Liu, Z. Y., & Gao, S. J. (2022). Acute myeloid leukemia with CEBPA mutations: current progress and future directions. Frontiers in Oncology, 12, 806137.
34. De la Torre, E. P., Serrano, J., Martínez-Cuadrón, D., Torres, L., Sargas, C., Ayala, R., Bilbao-Sieyro, C., Chillón, M. C., Larráyoz, M. J., Soria, E., Aparicio-Pérez, C., Bergua, J. M., Bernal, T., Gil, C., Tormo, M., Algarra, L., Alonso-Domínguez, J. M., Rodriguez-Arbolí, E., Martínez-Sanchez, P., Oliva, A., … Montesinos, P. (2024). Validation of mutated CEBPA bZIP as a distinct prognosis entity in acute myeloid leukemia: a study by the Spanish PETHEMAregistry. Haematologica, 109(8), 2682–2687.