RESEARCH ARTICLE

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The Non-Coding Code: Silent Regulators of *MEG3* and *Let-7i-3p/5p* in the Progression of Acute Lymphoblastic Leukemia

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Abstract

Objective: Acute lymphoblastic leukemia (ALL) is one of the most common malignancies worldwide. The long non-coding RNA *MEG3* functions as a tumor suppressor in several cancers, potentially influencing gene expression through transcriptional, translational, and epigenetic mechanisms. let-7i plays a role in leukemia progression. This study aimed to evaluate *MEG3* gene expression in adult ALL patients and investigate its possible regulatory interaction with let-7i-3p and let-7i-5p. **Methods:** A total of 83 blood samples, classified in a case-control study design, were collected from newly diagnosed ALL patients (n=53) and healthy controls (n=30). Hematological parameters were estimated using a CBC analyzer. RNA was extracted and reverse-transcribed into cDNA. Quantitative real-time PCR with gene-specific primers was employed to assess *MEG3* and let-7i-3p/5p expression levels. **Results:** ALL patients and healthy controls were matched for age (29.0±9.9 vs. 28.6±7.7 years, p=0.862) and sex (p=0.299). Hematological analysis revealed significant cytopenias in patients, including reduced Hemoglobin (Hb) (8.60±1.75 vs. 14.72±2.51 g/dL; p<0.001), white blood cells (WBCs) (4.91±2.84 vs. 7.96±1.57×10^9/L; p<0.001), and platelets (PLT) (156.96 ± 35.64 vs. 269.93±55.35×10^9/L; p<0.001). Gene expression analysis revealed that *MEG3* was significantly downregulated in ALL patients (fold change 0.535±0.273; p=0.001), whereas *Let-7i-3p/5p* were upregulated (fold changes 1.875 ± 0.732 and 1.857 ± 0.891: p=0.003 and 0.004, respectively), indicating a different dysregulation pattern associated with adult ALL. **Conclusion:** This study demonstrates that adult ALL patients exhibit hematological abnormalities including anemia, leukopenia, and thrombocytopenia. Molecular analysis revealed a significant downregulation of lncRNA *MEG3*, alongside upregulation of let-7i-3p/5p, suggesting their potential involvement in leukemogenesis. These findings highlight the diagnostic and possibly prognostic relevance of *MEG3* and let-7i in adult ALL and provide a basis for further investigation into their mechanistic roles and therapeutic targeting.

Keywords: *MEG3*- Let-7i-3p- Let-7i-5p- Acute lymphoblastic leukemia (ALL)- RT-PCR

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Introduction

Acute lymphoblastic leukemia (ALL) ranks as the second most common form of acute leukemia in adults, with more than 6,500 new cases diagnosed annually in the United States [1]. It is characterized by genetic mutations and chromosomal abnormalities that may disrupt the development and proliferation of lymphoid precursor cells, primarily of B-cell origin [2]. While risk stratification has usually been based on clinical indicators such as age and white blood cell count, the integration of genetic profiling has enhanced prognostic accuracy and informed treatment strategies [3]. Furthermore, elderly patients often struggle with treatment-related toxicity and face poorer prognoses [4].

Long non-coding RNAs (lncRNAs), which are transcripts longer than 200 nucleotides that do not encode proteins, have emerged as important regulators in various cellular processes and cancer development, including ALL [5]. While earlier studies mainly focused on protein-coding genes, recent research has shown that lncRNAs also display subtype-specific expression patterns and are associated with key clinical features such as diagnosis, prognosis, relapse, and treatment resistance in childhood ALL [6]. The lncRNAs are key regulators of cancer, modulating proliferation, apoptosis, angiogenesis, and metastasis through epigenetic, transcriptional, and post-transcriptional mechanisms [7]. Many act as competing endogenous RNAs (ceRNAs) that sponge microRNAs, and tumor-derived lncRNAs can also influence the

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microenvironment via exosomes, particularly by promoting angiogenesis [8]. Their cancer-specific expression and functional roles make lncRNAs important drivers of malignancy as well as potential biomarkers and therapeutic targets [9].

Long non-coding RNA maternally expressed gene 3 (Lnc-*MEG3*) is an imprinted gene located at the DLK1-*MEG3* locus on chromosome 14q32.3 and is widely expressed in various tissues, including the adrenal glands and liver [10]. Dysregulation of Lnc-*MEG3* has been shown to act as a tumor suppressor by interacting with target microRNAs and modulating cancer-related signaling pathways, such as the p53-dependent and Wnt/β-catenin pathways, particularly in hematological malignancies [11].

Let-7i belongs to the Let-7 family of microRNAs (miRNAs) located on chromosome12, which function as tumor suppressors in multiple types of cancer by targeting genes that drive tumor development [12]. Members of the Let-7 family, including Let-7i, regulate important genes involved in cell cycle control, cell proliferation, differentiation, and various signaling pathways, also exhibits a dual role, functioning to both promote and suppress different malignancies depending on its molecular targets [13]. The loss or downregulation of Let-7i can contribute to cancer progression by enabling the overexpression of oncogenes and factors associated with cellular stemness. In general, Let-7i had a vital role in decreasing tumor aggressiveness and lowering resistance to radiation and chemotherapy [14].

Recent investigations have highlighted the critical role of ceRNA networks in the pathogenesis of cancer, where lncRNAs, such as *MEG3*, operate as molecular sponges for miRNAs, thus regulating the expression of their downstream messenger RNA (mRNA) targets [15, 16]. This lncRNA's function as a ceRNA, specifically in sequestering miRNAs from their targets, can profoundly impact the gene expression programs driving malignancy, particularly because *MEG3* expression is characteristically lost or downregulated in human tumors, contrasting its presence in healthy tissues [16]. These observations provide the foundational rationale for assessing the expression profiles of *MEG* and both arms of Let-7i (the 3p and 5p isoforms) in ALL, especially considering the established tumor-suppressor function of *MEG3* and the regulatory capacity of Let-7i isoforms over genes crucial for cell proliferation, differentiation, and apoptosis, thus leading to the current study's design to evaluate *MEG3* expression in adult ALL patients and explore its potential regulatory interactions with Let-7i-3p and Let-7i-5p.

Materials and Methods

Ethical Approval

The study proposal, including its description and consent form, was reviewed and approved by the College of Science Research Ethics Committee at Mustansiriyah University (Approval ID: BCSMU/3035/00077M in January,1,2025), and all procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from the parents of all participants.

Sample Collection and Selection Criteria

A total of 53 adult acute lymphoblastic leukemia (ALL) patients and 30 age- and sex-matched healthy controls were initially recruited. The project will be conducted at the College of Science, Mustansiriyah University and the Iraqi Hereditary Company (IHC) with sample collection obtained from Baghdad Hospital at Medical City in Baghdad, Iraq, during the proposed period from 1 March 2025 to 30 May 2025. For the subsequent gene expression analysis, a subset of 30 ALL patients and 30 healthy controls was systematically selected. This selection process was based on established hematological parameters including hemoglobin (Hb), white blood cell count (WBC), and platelet count (PLT) to ensure representative sampling and minimize biological variability between the groups.

Venous blood samples (5 mL) were collected from each participant under aseptic conditions by trained healthcare personnel. Of this volume, (3 mL) was placed into EDTA-containing tubes for complete blood count (CBC) testing, while the remaining (2 mL) was reserved for subsequent nucleic acid extraction. The EDTA samples designated for molecular analysis were divided into two aliquots (one for miRNA extraction and the second for lnRNA analysis). Each aliquot consisted of 250 µL of whole blood immediately mixed with 750 µL of TRIzol reagent to preserve RNA integrity. All processed samples were stored at −80 °C until further analysis.

Inclusion criteria for patients include confirmed diagnosis of adult ALL, age ≥18 years, and availability of complete hematological data (Hb, WBC, PLT) while healthy controls were age- and sex-matched adults with normal hematological parameters and no history of hematological disorders. Exclusion criteria for both groups included children, presence of chronic infections, other malignancies and family history to malignancies.

Hematological Parameters

A comprehensive complete blood count (CBC) analysis was performed on a total of 83 participants using an automated hematology analyzer (Mindray Company, Germany). This test was conducted to assess a range of main hematological parameters, including total WBC, Hb concentration PLT count [17].

Molecular Study

Total RNA was isolated directly from whole blood samples using the TransZol Up Plus RNA Kit (ER501-01, TransGen Biotech, China) in while miRNA extraction was employed using EasyPure® miRNA Kit (ER601-01, TransGen Biotech, China) in accordance with the manufacturer's instructions [18]. The concentration and purity of the extracted RNA were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), ensuring that the RNA samples met quality requirements for downstream quantitative real-time polymerase chain reaction (RT-qPCR) analysis [19]. Concentration of extracted RNA was (28-41 ng/µL) and purity 2.0 to 2.2 according to A260/A280 ratio.

Complementary DNA (cDNA) synthesis was performed using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix kit (TransGen Biotech, China). The reaction mixture contained the provided reaction mix, random primers, anchored oligo(dT) primers, genomic DNA remover, RNase-free water, reverse transcriptase, and the extracted total RNA as the template. The synthesis process was carried out in a thermal cycler under the following conditions: initial incubation at 25 °C for 10 min, reverse transcription at 42 °C for 15 min, followed by enzyme inactivation at 85 °C for 5 s [20]. The primers used in this study were custom-designed using Primer 3 Plus and synthesized by Macrogen Company (Korea). Sequences were delivered in lyophilized form and reconstituted in nuclease-free water to a working concentration of 10 pmol/µL as described by [21]. The primer sequences and their details were description in Table 1.

Quantitative PCR amplification was carried out using the QIAGEN Rotor-Gene Q Real-Time PCR System (Qiagen, Germany) with the TransStart® Top Green qPCR SuperMix kit (TransGen Biotech, China) [22]. Gene expression levels and relative fold changes of *MEG3* and normalized using *GAPDH*, while *Let7i-3p* and *Let7i-5p* normalized by snRNA-U6 were determined based on the cycle threshold (Ct) values. PCR cycling conditions were adapted from a previously published protocol [23], with an annealing temperature of 62 °C for *MEG3*, 60 °C *GAPDH*, and 58 °C for each *Let7i-3p*, *Let7i-5p* and snRNA-U6.Gene expression fold change was calculated by the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Differences between groups were evaluated using the independent t-test, with p-values < 0.05 considered statistically significant. Receiver Operating Characteristic (ROC) curve analysis was performed to assess diagnostic accuracy, with area under the curve (AUC) values reported. Pearson's correlation was used to analyze relationships between variables, including corresponding p-values. All analyses were conducted using SPSS version 26 and MedCalc software version 23.3.2 [24, 25].

Results

Demographic and hematological parameters

The demographic and hematological characteristics of the study population are summarized in Table 2. The patient group comprised adults diagnosed with ALL, with a mean age of (29.0 ± 9.9 years), which is comparable to the control group (28.6 ± 7.7 years) with no significant difference (p = 0.862). This indicates that age was well-matched between ALL patients and healthy controls. Regarding sex distribution, 63.3% of patients were male and 36.7% female, while the control group included 46.7% males and 53.3% females and (p = 0.299), suggesting no statistically significant sex bias between groups.

Hematological parameters result indicates that patients with ALL exhibited reflective cytopenias compared to controls. Hb levels were highly significant lower in patients (8.60 ± 1.75 g/dL) than controls (14.72 ± 2.51 g/dL), reflecting the severe anemia commonly associated with marrow infiltration by leukemic blasts. Similarly, WBC counts were significantly reduced in patients (5.14 ± 2.81 × 10^9/L) versus controls (7.85 ± 1.66 × 10^9/L), which may reflect marrow suppression and disease heterogeneity in adult ALL. PLT counts were also significantly lower in patients compared to healthy individuals (156.96 ± 35.64 × 10^9/L, 269.93 ± 55.35 × 10^9/L; p < 0.001), consistent with thrombocytopenia resulting from impaired hematopoiesis.

Overall, these findings demonstrate that the patient cohort exhibits the classical hematological manifestations of adult ALL, including anemia, leukopenia, and thrombocytopenia, while age and sex were appropriately matched with the control group to minimize confounding variables.

Selection of an Optimal Reference Gene for MEG3 Quantification

NormFinder (https://moma.dk/normfinder-software) is a model-based approach for identifying the most stable normalization genes where a lower stability value indicates more consistent expression across all samples and experimental conditions. In this study, the suitability of *GAPDH* and snRNA-U6 as reference genes for lncRNA quantification was evaluated using the NormFinder algorithm to assess expression stability. Both genes demonstrated low expression variability, indicating potential utility for normalization in lncRNA RT-qPCR assays. Although snRNA-U6 exhibited slightly higher stability (stability score: 0.615) compared to *GAPDH* (stability score: 0.889), *GAPDH* was selected as the reference gene due to its widespread use and well-established reliability in lncRNA studies Table 3.

GAPDH is frequently preferred in gene expression analyses because of its consistently stable expression across diverse cell types and tissues, providing a dependable baseline for relative quantification. Its high transcript abundance enables precise detection of subtle changes in target gene expression, while its relative insensitivity to minor experimental variations reduces potential measurement bias. Moreover, its well-characterized sequence simplifies primer design and minimizes the risk of cross-reactivity, reinforcing its suitability as a reference gene.

Collectively, these characteristics, along with confirmation of its stability in the present experimental context, support the use of *GAPDH* as a reliable reference for accurate normalization in lncRNA expression studies. The observed stability scores indicate that a lower value reflects more consistent expression across samples; in this case, both *GAPDH* and snRNA-U6 showed low variability, validating their applicability, with the small difference in stability likely attributable to minimal variation between patient and control groups.

Relative Expression of MEG3and miR- Let7i-3p/5p

The qRT-PCR results for *MEG3*, *Let7i-3p* and *Let7i-5p* were display in Figure 1 and Figure 2. Figure 1 showed the amplification curve for study parameters including *MEG3*, *Let7i-3p* and *Let7i-5p* for detection Ct value while

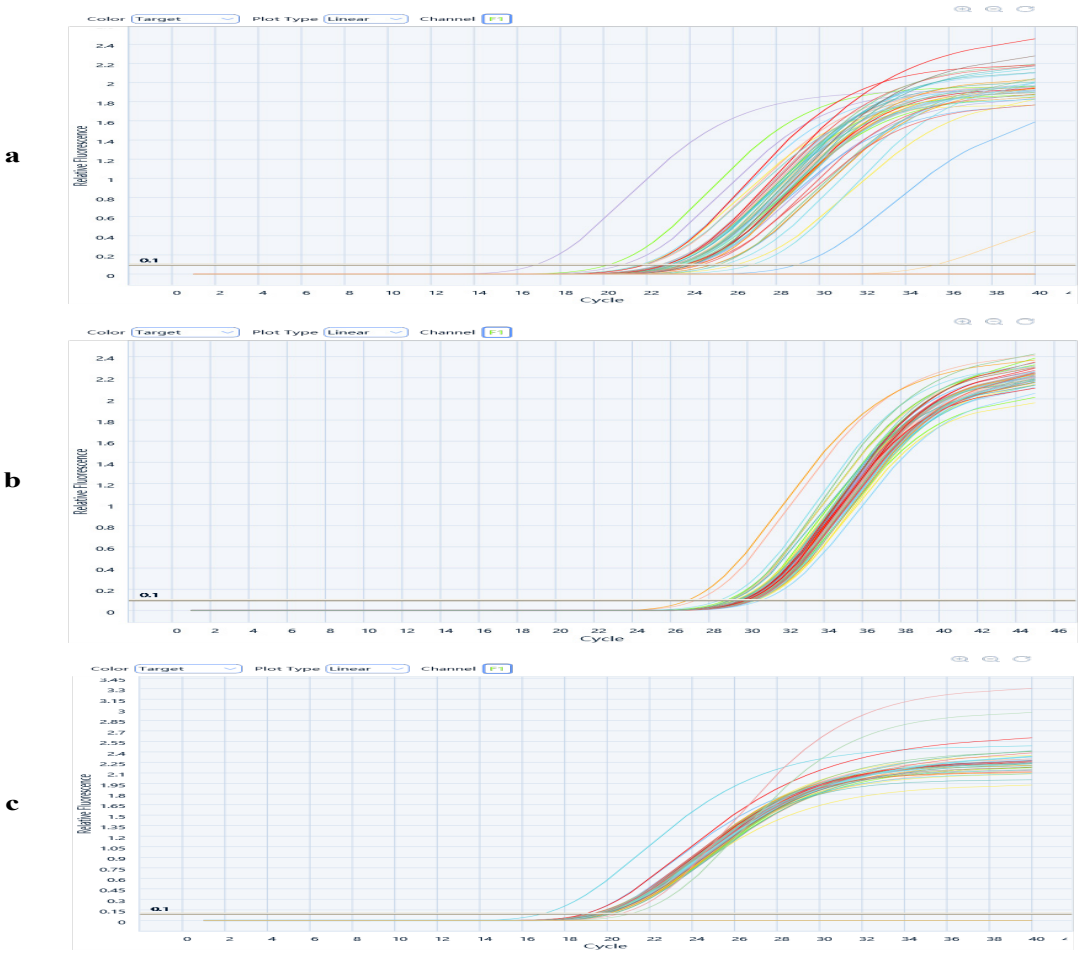

Figure 1. Real-time PCR Amplification Curves of Study Parameters. (a) *MEG3* gene, (b) *Let7i-3p*, (c) *Let7i-5p*

Figure 2 illustrates the disassociation curve respectively to ensure the primer binding site end exclude any primer dimer. The relative expression of *MEG3* and Let-7i (Let-7i-3p and Let-7i-5p) in ALL patients and healthy controls is summarized in Table 4, Figure 3. Expression levels were calculated using the ΔΔCt method, with *GAPDH* and snRNA-U6 serving as the housekeeping gene for normalization.

MEG3 was significantly downregulated in ALL patients, with a ΔCt of 1.340 compared to 0.042 in controls, corresponding to a ΔΔCt of 1.299 and a reduced fold expression of 0.535 ± 0.273 (p = 0.001). In contrast, Let-7i-3p and Let-7i-5p were upregulated: Let-7i-3p showed a ΔCt of 8.233 versus 9.036 in controls (ΔΔCt –0.803, fold change 1.875 ± 0.732, p = 0.003), while Let-7i-5p had a ΔCt of –1.613 versus 0.219 (ΔΔCt –1.832, fold change 1.857 ± 0.891, p = 0.004). Together, these results demonstrate a clear differential expression pattern in adult ALL, with *MEG3* downregulation and Let-7i upregulation, highlighting their potential roles as diagnostic and therapeutic biomarkers.

ROC Curve Analysis for Three Parameters

Receiver Operating Characteristic (ROC) curves

Table 1. Primer Sequences and Their Details for *MEG3, Let7i-3p, Let7i-5p* and Housekeeping Genes

Gene	Direction	Sequence	Tm (°C)	Ta (°C)
MEG3	Forward	CTGCCCATCTACACCTCACG	63.2	62
	Reverse	CTCTCCGCCGTCTGCGCTAGGGGCT	75.7	
GAPDH	Forward	GGCCTCCAAGGAGTAAGACC	59.46	60
	Reverse	AGGGGTCTACATGGCAACTG	59.38	
Let7i-3p	Forward	CTGCGCAAGCTACTGCCTTGCT	65.4	58
Let7i-5p	Forward	TGAGGTAGTAGTTTGTGCTGTT	57	58
SnRNA-U6	Forward	AGAGAAGATTAGCATGGCCCCT	60	58
*MiR-R.P**	Reverse	GCGAGCACAGAATTAATACGAC	62	-
Universal miR	-	CAGGTCCAGTTTTTTTTTTTTTTVN	-	-

* MiR-R.P: MiRNA-universal Reverse primer.

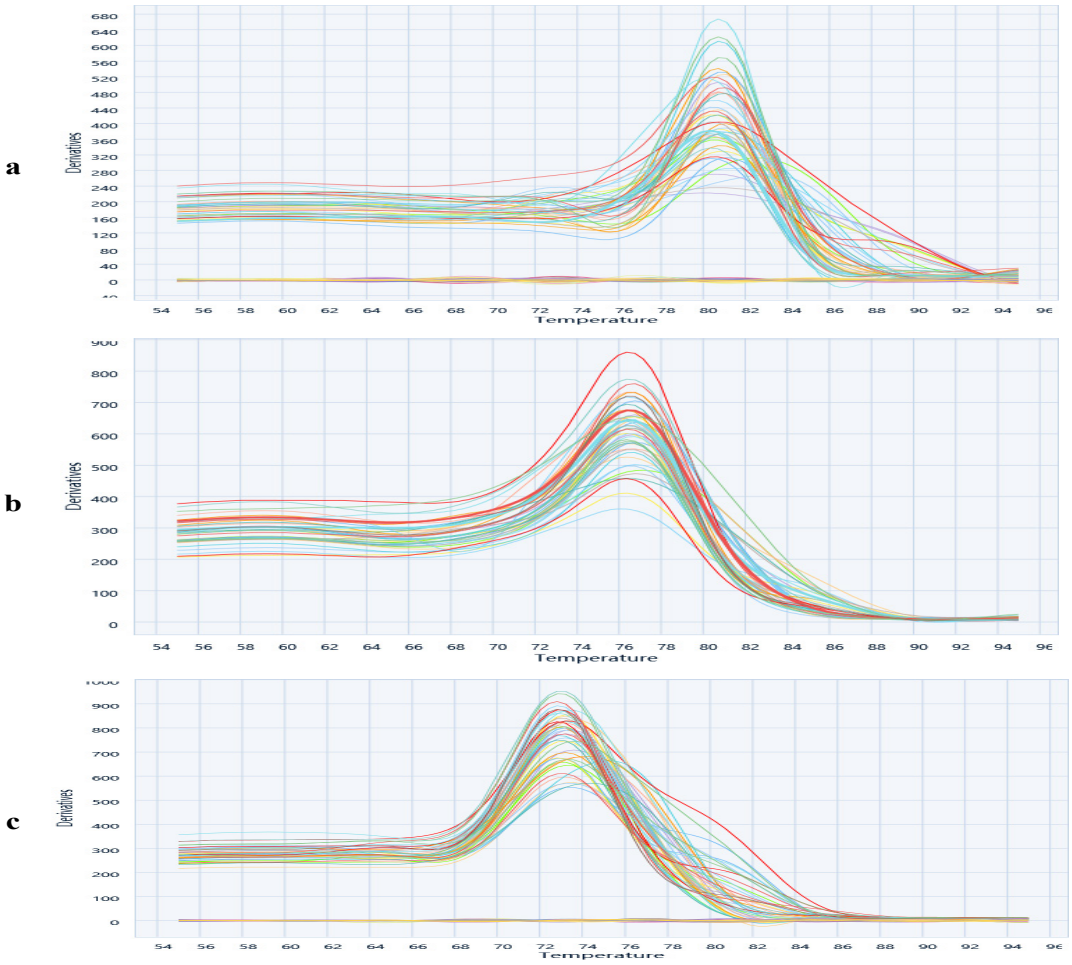

Figure 2. Real-Time PCR Disassociation Curves of Study Parameters. (a) *MEG3* gene, (b) *Let7i-3p*, (c) *Let7i-5p*

was generated for the three studied parameters (*MEG3*, *Let-7i-3p* and *Let-7i-5p*) to evaluate their discriminative ability between cases and controls. The area under the curve (AUC) with 95% confidence intervals (CI) was calculated using the DeLong method, and the optimal cutoff point for each parameter was determined according

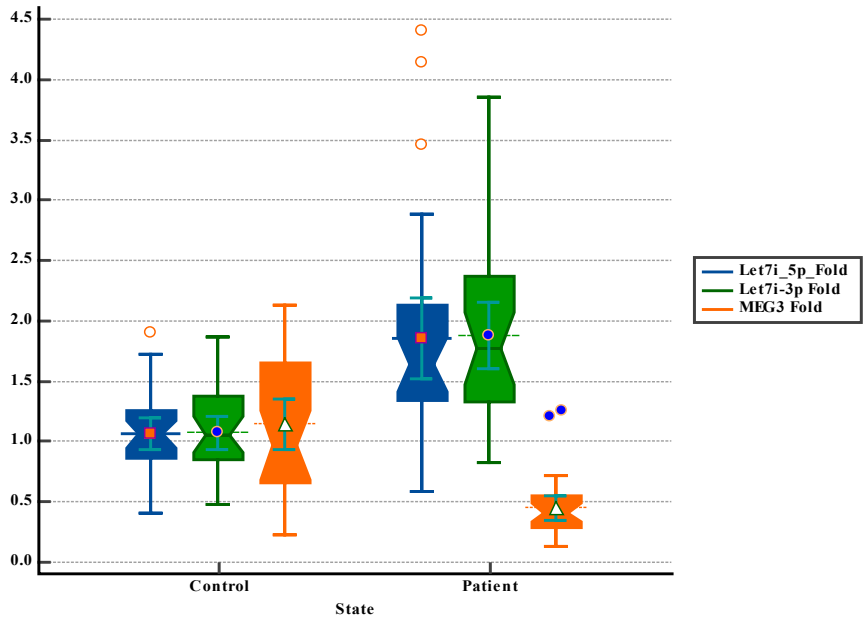

Figure 3. Notched Boxplots to Visualize Gene Expression Fold Changes (lncRNA MEG3 and miR-Let-7i (3p and 5p) in Two Groups: Control (left) and ALL Patient (right). The color of each boxplot in the figure corresponds to a specific gene: blue represents Let7i-5p Fold, green represents Let7i-3p Fold, and orange represents MEG3 Fold, as indicated by the legend on the right side of the figure.

Table 2. Demographic and Hematological Characteristics of Adult ALL Patients and Healthy Controls

Parameters	Group	Mean ± SD or Count (%)	p- value
Age (Year)	Patients	29.00 ± 9.92	0.862 NS
	Control	28.60 ±7.70	
Sex			
Male	Patients	19 (63.33%)	0.299 NS
	Control	14 (46.67%)	
Female	Patients	11 (36.67%)	
	Control	16 (53.33%)	
Hb (g/dL)	Patients	8.60 ± 1.75	0.001**
	Control	14.72 ± 2.51	
WBC (*10^9L)	Patients	5.14 ± 2.81	0.001**
	Control	7.85 ± 1.66	
PLT (*10^9L)	Patients	156.96 ± 35.64	0.001**
	Control	269.93 ± 55.35	

to Youden's index. Performance measures at both the optimal threshold and at clinically relevant "rule-out" and "rule-in" cutoffs are reported, including sensitivity, specificity, likelihood ratios (LR+ and LR−), and predictive values (PPV and NPV).

Table 3. Stability and Variation of Reference genes *GAPDH* and snRNA-U6 for *MEG3*

Parameters	*GAPDH*	*U6*
Stability	0.899	0.615
Variation within one group	0.193	0.034
Variation between Patients &control	0.707	0.581

MEG3 demonstrated strong diagnostic performance, with an AUC of 0.884 (95% CI: 0.776–0.953, $p < 0.001$). Using the optimal Youden cutoff of ≤0.603, it achieved 83.33% sensitivity and 80.00% specificity, corresponding to LR+ = 4.17, LR− = 0.21, PPV = 88.1%, and NPV = 73.1%, indicating solid utility for both ruling in and ruling out disease. Let-7i-3p also showed very good discriminative ability, with an AUC of 0.840 (95% CI: 0.723–0.922, $p < 0.001$). At the cutoff >1.44, sensitivity and specificity were 70% and 90%, respectively (LR+ = 7.00, LR− = 0.33); while its PPV was high, the lower NPV suggests reduced reliability for confirming healthy status. Let-7i-5p demonstrated comparable performance with an AUC of 0.829 (95% CI: 0.710–0.914, $p < 0.001$). The optimal cutoff (>1.257) yielded 86.3% sensitivity and 72.2% specificity, with LR+ = 3.57, LR− = 0.22, PPV = 86.3%, and NPV = 72.2%. Table 5 and Figure 4 present the full ROC analysis.

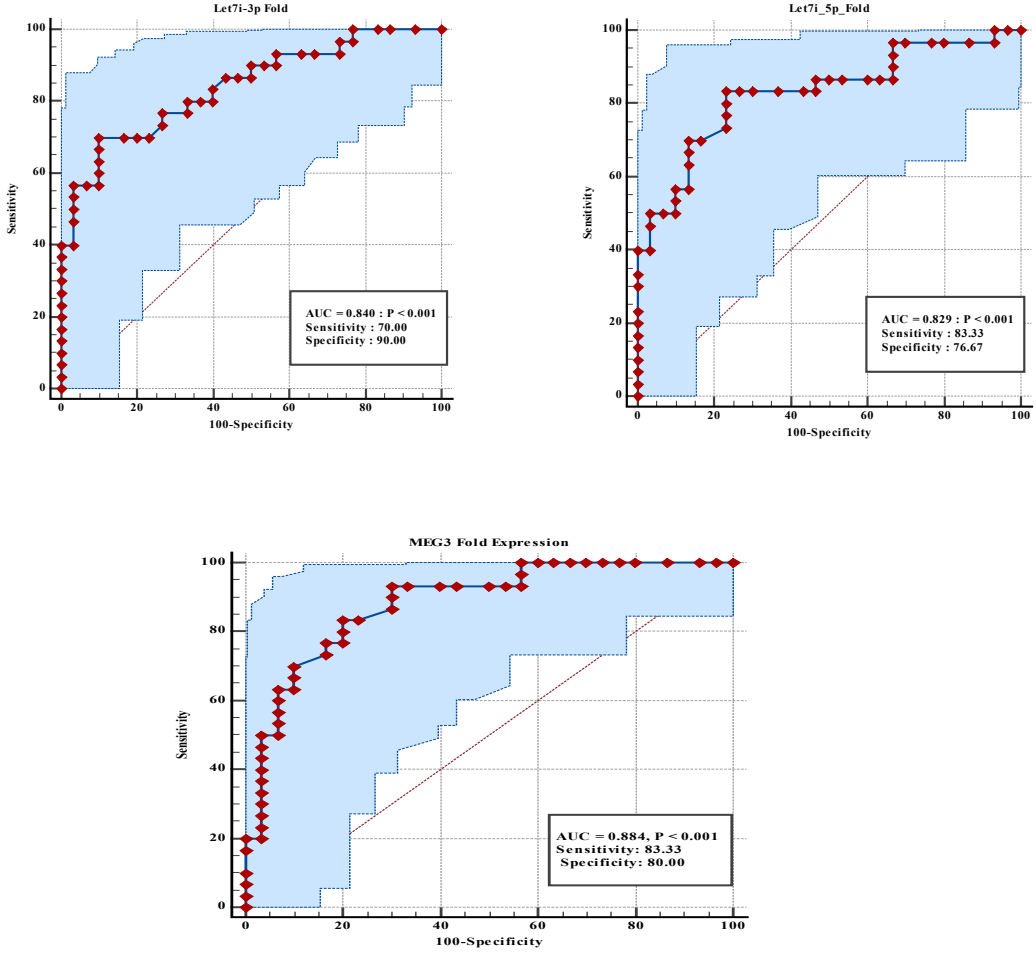

Figure 4. Receiver Operating Characteristic Curves Illustrating the Diagnostic Accuracy of the Three Evaluated Parameters. The curves display the trade-off between sensitivity and specificity, and the AUC provides a quantitative measure of diagnostic performance.

Table 4. Differential expression of *lncRNA MEG3* and *miR- Let-7i* (3p and 5p) in ALL Patients Compared to Controls

Groups	TG (Mean Ct)	HKG (Mean Ct)	ΔCt	ΔΔCt	Fold ($2^{-\Delta\Delta Ct}$)	p- value
MEG3 Fold						
Patients	23.613	22.273	1.34	1.299	0.535 ± 0.273	0.001**
Control	23.192	23.151	0.042	-0.00003	1.126 ± 0.561	
Let7i-3p Fold						
Patients	29.794	21.561	8.233	-0.803	1.875 ± 0.732	0.003**
Control	30.227	21.192	9.036	0	1.072 ± 0.379	
Let7i-5p Fold						
Patients	19.815	21.428	-1.613	-1.832	1.857 ± 0.891	0.004**
Control	19.735	19.516	0.219	-2.3E-16	1.066 ± 0.363	

Pairwise comparison of ROC curves

Pairwise comparisons using the DeLong test indicated that *MEG3*_Fold did not significantly outperform Let7i_3p_Fold (ΔAUC = 0.044, 95% CI: -0.088 to 0.177, p = 0.510) or Let7i_5p_Fold (ΔAUC = 0.056, 95% CI: -0.080 to 0.191, p = 0.422). Similarly, Let7i_3p_Fold and Let7i_5p_Fold showed no significant difference in discriminative ability (ΔAUC = 0.011, 95% CI: -0.104 to 0.126, p = 0.850). These results suggest that none of the three markers demonstrated superior diagnostic performance in this dataset. Although individual comparisons were not significant, combining *MEG3* fold with Let7i fold parameters in a multivariable model could potentially improve overall discrimination by capturing complementary information. Table 6 and Figure 5 showed the result of Delong test.

Correlation test among study parameters

Pearson correlation analysis demonstrated significant associations between *MEG3*, Let-7i isoforms, and hematological parameters. *MEG3* expression showed a strong positive correlation with hemoglobin (r = 0.607, p < 0.01) and platelet counts (r = 0.544, p < 0.01), and a moderate positive correlation with white blood cell counts (r = 0.351, p < 0.01). Conversely, Let-7i-3p and Let-7i-5p exhibited negative correlations with these parameters, with Let-7i-3p strongly negatively associated with Hb (r = -0.492, p < 0.01) and WBC (r = -0.489, p < 0.01), and moderately with PLT (r = -0.377, p < 0.01). *MEG3* also negatively correlated with Let-7i-3p (r = -0.370, p < 0.01) and Let-7i-5p (r = -0.335, p < 0.01). Age did not show significant correlations with any parameter (r = -0.07 to -0.09, p > 0.05). Table 7 explains the results.

These findings suggest that *MEG3* may play a supportive role in maintaining hematological homeostasis, as indicated by its positive association with Hb, WBC, and PLT levels. In contrast, the negative correlations of Let-7i-3p and Let-7i-5p with these parameters imply a potential suppressive effect on hematopoiesis. The inverse relationship between *MEG3* and Let-7i isoforms further

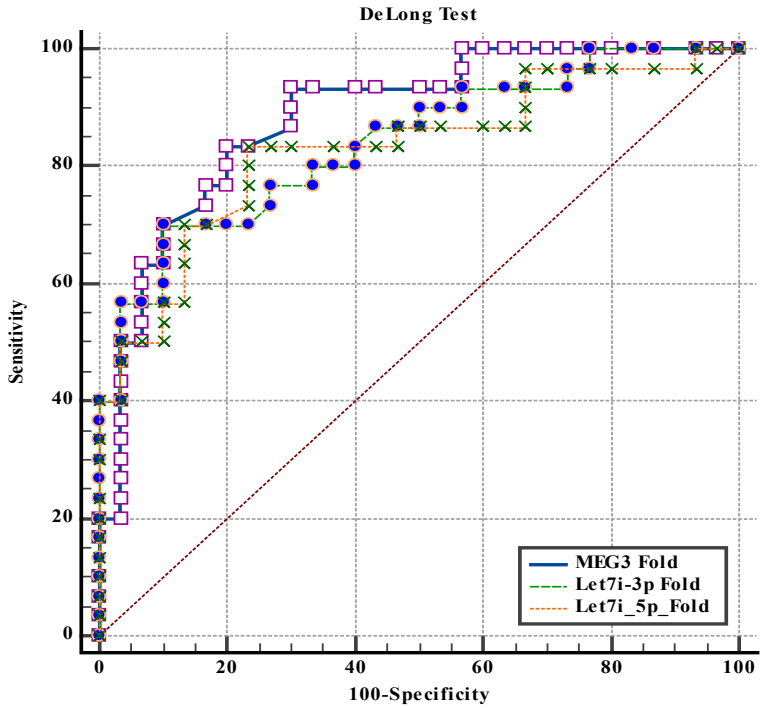

Figure 5. The DeLong Test for ROC Curves Comparison for Study Parameters Including *MEG3* Fold, *Let7i-3p* Fold, and *Let7i-5p* Fold demonstrating similar discriminative abilities, with no significant differences.

Table 5. ROC Curve Analysis for the Three Evaluated Parameters, Including Sensitivity, Specificity, AUC, Likelihood Ratios, and Predictive Values

Test	*MEG3*	*Let-7i-3p*	*Let-7i-5p*
Area under the ROC curve (AUC)	0.884	0.84	0.829
Standard Error [a]	0.043	0.051	0.054
95% Confidence interval [b]	0.776 to 0.953	0.723 to 0.922	0.710 to 0.914
95% Bootstrap CI [c]	0.774 to 0.951	0.706 to 0.920	0.692 to 0.918
z statistic	8.928	6.685	6.049
Significance level P (Area=0.5)	<0.0001	<0.0001	<0.0001
Youden index J	0.633	0.6	0.6
95% Confidence interval [a]	0.4000 to 0.7667	0.3667 to 0.7667	0.3667 to 0.7667
Cut off	≤0.603	>1.44	>1.257
95% Confidence interval [a]	≤0.451 to ≤0.717	>1.137 to >1.7	>1.24 to >1.906
Sensitivity	83.33	70	83.33
Specificity	80	90	76.67
Positive Likelihood Ratio (LR+)	4.17	7	3.57
Negative Likelihood Ratio (LR−)	0.21	0.33	0.22
Positive Predictive Value (PPV)	0.881	0.925	0.863
Negative Predictive Value (NPV)	0.731	0.629	0.722

Table 6. Pairwise Comparison of ROC for *MEG3, Let7i-3p* and *Let7i-5p* Folds Expression Using the DeLong Test.

MEG3_Fold ~ Let7i_3p_Fold	
Difference between areas	0.0444
Standard Error	0.0675
95% Confidence Interval	-0.0878 to 0.177
z statistic	0.659
Significance level	P = 0.5099
MEG3_Fold ~ Let7i_5p_Fold	
Difference between areas	0.0556
Standard Error	0.0692
95% Confidence Interval	-0.0800 to 0.191
z statistic	0.803
Significance level	P = 0.4218
Let7i_3p_Fold ~ Let7i_5p_Fold	
Difference between areas	0.0111
Standard Error	0.0587
95% Confidence Interval	-0.104 to 0.126
z statistic	0.189
Significance level	P = 0.8499

suggests an antagonistic regulatory interplay, which may be biologically relevant in conditions such as acute lymphoblastic leukemia. Age-independent correlations indicate that these gene expression effects are not significantly influenced by patient age in this investigation. Overall, the data highlight a potential functional axis between *MEG3* and Let-7i that could modulate blood cell production and disease progression.

Discussion

This study represents the first investigation in Iraq to evaluate the regulatory interplay between lncRNA *MEG3* and both strands of miR-let-7i (5p and 3p) in ALL. Although previous studies have assessed *MEG3* expression in leukemia or examined individual members of the let-7 family, none have simultaneously explored *MEG3* alongside the dual-arm behavior of let-7i. This integrated approach offers a novel perspective on the molecular mechanisms underlying ALL and lays the groundwork for future research. Clinically, most patients presented with pancytopenia at diagnosis (manifesting as anemia, thrombocytopenia, and variable WBC counts ranging from leukopenia to marked leukocytosis) aligning with established ALL presentation patterns [26]. These hematologic disturbances reflect the extent of bone marrow failure and provide essential clinical context for understanding the involvement of *MEG3* and miR-let-7i strands in leukemogenesis.

Our findings indicate that decreased *MEG3* levels in ALL patients are associated with elevated *Let-7i*-3p and *Let-7i*-5p, supporting a ceRNA model in which *MEG3* acts as a molecular sponge for *Let-7i* [27]. Loss of *MEG3* may enhance *Let-7i* activity, promoting cancer cell growth by suppressing tumor suppressor genes, supporting cell survival, and reducing programmed cell death [28]. However, the causal relationship requires validation through functional experiments such as overexpression or knockdown of *MEG3* and *Let-7i*, alongside mechanistic assays including RNA immunoprecipitation. Investigating their subcellular localization and transcriptional or epigenetic regulation could provide deeper insights into the underlying molecular mechanisms. These findings offer an important framework for understanding the role of *MEG3* and *Let-7i* in ALL progression and may open new avenues for potential therapeutic strategies [13, 14,

Table7. Pearson Correlation Coefficients between *MEG3, Let-7i* Isoforms, and Blood Parameters

Parameters	Pearson Correlation (r)	p-value	Interpretation of Value
MEG3 vs Hb	0.607**	$p < 0.01$	Strong positive correlation
MEG3 vs WBC	0.351**	$p < 0.01$	Moderate positive correlation
MEG3 vs PLT	0.544**	$p < 0.01$	Strong positive correlation
MEG3 vs Let7i-3p	-0.370**	$p < 0.01$	Negative correlation
MEG3 vs Let7i-5p	-0.335**	$p < 0.01$	Negative correlation
Let7i-3p vs Hb	-0.492**	$p < 0.01$	Strong negative correlation
Let7i-3p vs WBC	-0.489**	$p < 0.01$	Strong negative correlation
Let7i-3p vs PLT	-0.377**	$p < 0.01$	Moderate negative correlation
Let7i-5p vs Hb	-0.415**	$p < 0.01$	Negative correlation
Let7i-5p vs WBC	-0.457**	$p < 0.01$	Negative correlation
Let7i-5p vs PLT	-0.333**	$p < 0.01$	Moderate negative correlation
Age vs all parameters	-0.07 to -0.09	$p > 0.05$	No significant correlations

29, 30].

The findings from this study are consistent with and build upon a growing body of research on the role of lncRNAs in cancer. Previous studies have similarly identified lnc-*MEG3* as a tumor suppressor in various malignancies. For instance, research has shown that lnc-*MEG3* expression is often downregulated in cancer cells, and its overexpression can inhibit cell proliferation, migration, and invasion while promoting apoptosis [36]. Also studies have demonstrated that *MEG3* is downregulated in ALL and other malignancies, where its loss promotes tumor progression. Mechanistically, *MEG3* can act as a molecular sponge for miR-9-5p in hepatocellular carcinoma (HCC), thereby regulating tumor suppressors like SOX11[31]. Another clinical and molecular studies consistently demonstrate that *MEG3* expression is significantly reduced in papillary renal cell carcinoma (PRCC) [32], non-small cell lung cancer (NSCLC) [33] and bladder cancer [34], where its loss is strongly associated with enhanced tumor cell proliferation, migration, invasion, impaired apoptosis, and poor overall survival.

Previous studies suggest that Let-7i may act as a tumor suppressor in ALL and serve as a treatment-response indicator, while other Let-7 family members inhibit proliferation and promote differentiation in AML. Moreover, reduced Let-7i expression has been linked to treatment resistance in CML. However, the specific role of Let-7i in ALL remains unclear, as its expression is infrequently elevated compared with AML, where it shows higher levels. In our study, we observed a distinct expression pattern of Let-7i in leukemia, which may be shaped by regulatory interactions with long non-coding RNAs that directly modulate its function. These findings highlight the need to clarify the biological and clinical relevance of Let-7i-3p and Let-7i-5p in ALL [14]. The current study showed elevated Let-7i expression in ALL patients, that are in line with a recent study in clear cell renal cell carcinoma (ccRCC), where Let-7i-5p was found to promote cell proliferation, migration, and invasion by directly targeting the tumor suppressor HABP4. This parallel across distinct malignancies suggests that Let-7i may not act solely as a tumor suppressor, as previously reported, but rather as a context-dependent oncogenic regulator. Accordingly, the elevated Let-7i observed in ALL in our cohort may contribute to enhanced leukemic cell viability and treatment resistance, highlighting the need for further functional investigations to identify its direct molecular targets in leukemia [35]. In summary, decreased *MEG3* expression in ALL may elevate Let-7i-3p and Let-7i-5p through a ceRNA-mediated mechanism. This aligns with evidence from other cancers where *MEG3* functions as a molecular sponge for miRNAs, such as miR-421 in breast cancer [36] and miR-181 in gastric cancer [37]. Additionally, Let-7i has been shown to suppress oncogenes including HMGA2 and MYC, underscoring its context-dependent role in tumorigenesis [38]. Collectively, these findings support a potential *MEG3*–Let-7i regulatory axis in ALL and highlight the need for functional validation through molecular assays. From a translational standpoint, integrating the expression profiles of *MEG3* and both Let-7i arms with clinical variables such as age, hemoglobin, white blood cell count, and platelet count may enhance diagnostic precision and prognostic stratification in ALL. Patients presenting with noticeable low hemoglobin and platelet levels, together with reduced *MEG3* expression and aberrant Let-7i activity, may constitute a high-risk subgroup characterized by poor treatment response. These observations highlight the therapeutic potential of RNA-based strategies, either through restoration of *MEG3* expression or selective modulation of Let-7i isoform activity, as promising approaches for improving clinical outcomes.

Study Limitations

Despite providing novel insights, this study has several limitations. The relatively small sample size reduces statistical power and limits the generalizability of our findings. Functional validation experiments, such as knockdown or overexpression models, were not performed to confirm the mechanistic interactions between *MEG3* and let-7i arms. In vivo studies and longitudinal monitoring of hematologic parameters were also lacking, restricting our ability to correlate gene expression with

treatment response over time. Furthermore, although a ceRNA-based mechanism was proposed, molecular validation experiments are required to demonstrate the direct interaction between *MEG3* and let-7i. Finally, financial constraints and self-funding limited the scope and scale of the experimental work.

In conclusion, the current study reveals a significant downregulation of *MEG3* and upregulation of miR-let7i-3p/5p in adults with acute lymphoblastic leukemia, highlighting their complementary roles in leukemogenesis. RT-qPCR analysis confirmed these expression patterns, while ROC curve analysis demonstrated strong diagnostic potential for *MEG3* and both Let-7i isoforms (all AUCs >0.82, $p < 0.001$), suggesting their combined use could enhance diagnostic accuracy. Correlation analyses indicated positive associations between *MEG3* and hematological parameters, whereas Let-7i isoforms showed inverse correlation, reflecting an antagonistic regulatory interaction. Overall, these findings underscore the utility of *MEG3* and Let-7i-3p/5p as diagnostic and prognostic biomarkers in adult ALL and introduce a novel *MEG3*–Let-7i regulatory axis in disease pathogenesis. Further studies with larger cohorts and functional validation are warranted to confirm these targets and explore their potential in RNA-based therapies.

Author Contribution Statement

All researchers made equal contributions to every stage of the study, including its design, implementation, data analysis, and preparation of the final manuscript. All authors have reviewed and approved the final version of the manuscript and take full responsibility for the accuracy and integrity of the work.

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Conflicts of Interest

None. Authors confirm that all the Figures and Tables in the manuscript belong to this investigation.

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