Science Journal of University of Zakho
DOI: https://doi.org/10.25271/sjuoz

Original Article

EXPLORING THE ONCOGENIC AND TUMOR SUPPRESSIVE FUNCTIONS OF hsa-miR-125B AND hsa-miR-574 IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Received: 04, Jan, 2025
Accepted: 12, Aug, 2025
Published: 12, Apr, 2026

ABSTRACT

Acute lymphoblastic leukemia (ALL) is the second common type of leukemia. In leukemia, small non-coding RNAs (miRNAs) role as either tumor suppressors or oncomiRNAs. Evidence indicates an association between the dysregulation of these miRNAs and the progression and spread. An essential regulator in carcinogenesis and progression, hsa-miR-574 is up- or down-regulated by functional pathways, underlying new mechanisms. ALL prognosis and chemoresistance have both been linked to hsa-miR-125b.
Quantitative real-time polymerase chain reaction was employed to analyses the expression levels of hsa-miR-125b and hsa-miR-574 in 25 ALL patients (new and follow-up cases) and 20 healthy controls.
The findings indicated that the expression levels of hsa-miR-125b and hsa-miR-574 are modified in ALL, and their expression levels were markedly changed in ALL patients. The hsa-miR-125b expression level was upregulated in new cases and downregulated in follow-up cases. Conversely, hsa-miR-574 was downregulated in ALL new cases compared to controls. However, these levels increased in follow-up cases undergoing chemo-immunotherapy. The statistics derived from the ROC curve indicate that hsa-miR-125b expression in new cases had an AUC = 0.762, in follow-up cases had an AUC = 0.562, while the hsa-miR-574 expression in new cases had an AUC = 0.542 and in follow-up cases with an AUC = 0.916 were correlated with the ALL instances.
The hsa-miR-125b upregulation observed in ALL suggests its potential oncogenic role. Conversely, the downregulation of hsa-miR-574 predicts a possible tumor-suppressive function, given its reduced expression. These findings highlight the significance of hsa-miR-125b and hsa-miR-574 as key regulatory molecules in ALL progression.

KEYWORDS: Acute lymphoblastic leukemia, hsa-miR-574, hsa-miR-125b, bisphenol A.

1. INTRODUCTION

The majority common subtype of acute leukemia in children is acute lymphoblastic leukemia (ALL) (Muhsin *et al*., 2024), accounting for roughly 80–85% of global incidence (Tebbi, 2021; Terwilliger & Abdul-Hay, 2017). ALL predominantly affects children; however, in elderly patients, it is associated with a worse prognosis and is considered high-risk ALL, which is characterized by malignant proliferation of lymphoid progenitors that displaces normal hematopoiesis (Mendiola-Soto *et al*., 2023). Although treatments have raised survival rates, the treatment-related toxicity is still a major issue both in the short and long term. This underscores the significance of novel therapeutic strategies to treat low-risk as well as high-risk forms of ALL more efficiently. Currently, investigating molecules that have a role in the processes that cause leukemogenesis and its progression is crucial for identifying potential targets at the transcriptional level. MicroRNAs (miRNAs) are an essential class of endogenous tiny non-coding RNAs, measuring 19 to 25 nucleotides in length, transcribed by RNA polymerase II (Pol II) in the nucleus. They are essential in modulating signaling pathways related to lymphopoiesis and influence the differentiation and proliferation of hematopoietic cells (García-Alcocer & Becerra, 2020).

Varying genetic subtypes of ALL possess distinct miRNA expression profiles that indicate oncogenic activity, demonstrate tumor suppressive function, and are unequivocally associated with hematological malignancies, including leukemia (Bousquet *et al*., 2010). The over-expression of hsa-miR-125b has been linked to medication resistance in young ALL (Gefen *et al*., 2010; Schotte *et al*., 2012). Moreover, hsa-miR-125b is elevated in B-cell ALL patients exhibiting translocation in t(11; 14). (q24; q32) (Bousquet *et al*., 2010). Numerous investigations have focused on hsa-miR-125b, highlighting its potential oncogenic or tumor suppressive functions. hsa-miR-125b is consequent from the identical mature sequence as hsa-miR-125b-2, which is encoded by two distinct genes, MIR125B1 (11q24.1) and MIR125B2 (21q21.1). The expression abnormalities in inherited hsa-miR-125b have been associated with numerous solid and hematological tumors. The hsa-miR-125b has been shown to perform a function in lymphoblastic leukemia by targeting the IRF4 tumor suppressor, crucial for the maturation of B- and T-

https://doi.org/10.25271/sjuoz.2026.14.2.1632
Printed ISSN 2663-628X;
Electronic ISSN 2663-6298
Science Journal of University of Zakho
Vol. 14, No. 02, pp. 316 –326 April-2026

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cells. ARID3a enhances the proliferation of progenitor B-cells and elevates the encoding of pluripotency-linked transcription factors, including OCT4, SOX2, KLF4, and NANOG (Liu *et al*., 2016). Furthermore, hsa-miR-125b downregulates TNFAIP3, causing NF-κB-mediated enhancement of B-cell spread and a cease of apoptosis in disperse large B-cell lymphoma (DLBCL), alongside the inhibition of T-cell differentiation and metabolic reprogramming in ALL (Piatopoulou *et al*., 2017). The initial intron of the protein-coding gene FAM114A1 on human chromosome 4p14 contains the intronic miRNA gene MIR574, which codes deuce miRNAs: miR-574-5p and its complementary strand miR-574-3p. miR-574-5p is frequently among the most dysregulated microRNAs across diverse physiological and pathological conditions. Evidence demonstrates its pivotal role in post-transcriptional gene regulation (Yang, 2024).

This work summarizes the current evidence on the relationship between miRNAs and lymphopoiesis, as well as the development and progression of ALL, to propose and examine innovative ways for modulating ALL-associated miRNA levels as potential biomarkers.

2. MATERIALS AND METHODS

Collecting the study's samples:

This study included 25 consecutive treatment-naive patients diagnosed with ALL (25 new cases and 25 follow-up cases) at Nanakali Hospital for Cancer and Blood Diseases and 20 healthy volunteers. Peripheral blood samples were obtained from all participants, and serum was separated by centrifugation at 4000 rpm for 10 minutes at 4°C. Blood and serum samples were aliquoted and refrigerated at -80°C until further analysis. Clinical data were collected from all patients.

Quantification of serum biomarkers using ELISA:

Two milliliters of peripheral blood were obtained from all patients, including those with newly diagnosed conditions and follow-up cases, and healthy volunteers. Serum was separated through centrifugation at 350 × g for 10 minutes.

Quantitative RT-PCR for expression detection of miRNAs:

Total RNA was extracted utilizing the Total RNA Extraction Kit following the manufacturer's guidelines. The RNA quality was assessed with a Nanodrop spectrophotometer (Biometrics, Wilmington, USA) by determining the absorbance ratio at 260/280 nm. The synthesis of cDNA of miRNAs (utilizing specific stem-loop primers in RT of miRNA) was analyzed by performing the cDNA Synthesis Kit, following the manufacturer's instructions, focusing on two specific miRNAs (hsa-miR-125b and hsa-miR-574) performing the thermal cycling with priming at 25°C for 10 min, reverse transcriptase at 50°C for 60 min, RT inactivation at 80°C for 5 min, and finally holding at 12°C. Unique RT primers were utilized to evaluate the expression levels of both miRNAs, as revealed in Table 1. The qRT-PCR experiments were performed to assess miRNA expression by applying the SYBR green method, subsequent the manufacturer's guidelines (AddScript 2×SYBR Master) (AddBio Com, Korea) and qRT-PCR CFX96 (Bio-Rad, USA). A specific forward and universal reverse primer for both miRNAs was used in the qRT-PCR cocktail. The primer sequences applied in this research were incorporated into each PCR tube, with a 20 µL volume for the RT-PCR process. The cocktail was: 5 µL of cDNA template; 3 µL of nuclease-free water; 1.0 µL of primers with 10 pmol/µL; and 10 µL of SYBR Green Master Mix (without ROX). The PCR programs for both miRNAs and the internal controls was 95°C for 3 min, following 40 cycles of denaturation at 95°C for 35 sec, annealing (56.6°C for hsa-miR-125b, 54°C for hsa-miR-574, and 55°C for U6) for 40 sec, extension at 72°C for 45 sec, and the final extension step was performed one cycle at 72°C for 5 min. The C_T values for the samples were normalized to SNU6 expression as an internal reference, and the data were then converted into relative fold change using the $2^{-\Delta\Delta CT}$ algorithm.

Table 1: Oligonucleotide primers used in qRT-PCR for miRNA expression.

miRNAs	Primer status	Primer Sequence	Length of oligonucleotides (bps)	References
hsa-miR-574	Stem-loop	5′GTGTGTCGCTCCGGGTCCACGCTCATGCACAC ACCCACACGCCCACACTCAGGACACAC3′	59	Accession MI0000446
	Forward	5′GTTTCCGTGAGTGTGTGT3′	18	
	Reverse	5′CTCCGGGTCCACGCTCAT3′	18	miRBase entry:
hsa-miR-125b	Stem-loop	5′GTGTGTCGCTCCGGGTCCACGCTCATGCACAC ACCCACACGCCCACACTCAGGTCACAA3′	59	hsa-mir-125b-1
	Forward	5′CTCCGGGTCCACGCTCAT3′	22	Accession
	Reverse	5′TCCCTGAGACCCTAACTTGTGA3′	18	MI0003581
SNU6	U6-RT	5'CGCTTCACGAATTTGCGTGTCAT3'	23	
	Forward	5'GCTTCGGCAGCACATATCTAAAAT3'	23	miRBase entry:
	Reverse	5'CGCTTCACGAATTTGCGTGTCAT3'	23	hsa-mir-574

Immunophenotyping through flow cytometry:

Cases of Acute Lymphoblastic Leukemia (ALL) were categorized according to the results of flow cytometry conducted at the Nanakali Hospital for Blood Diseases and Cancer in Erbil, Iraq.

Performing multicolor flow cytometry and marker panels, the ALL data were obtained from the physician reports for the present work. Achievements were performed on a BD FACSCanto II flow cytometer (BD Biosciences) and investigated with FlowJo software version 10.9.0 (TreeStar Inc., San Francisco, CA, USA). Moderate to intense CD45 expression with low side scatter, cCD3+, small CD3+, TdT+, CD34, CD10+, CD2+, CD5+, CD7+, CD4-, CD8+ indicates a profile consistent with T-ALL. Dim or negative CD45 expression with low SSC, CD19+, CD10+ (moderate and heterogeneous), HLA-DR+, cCD79a+, cIgM, CD2-, CD33-, cCD3-, and MPO- presented a indicative profile of B-ALL. Following the harmonized flow cytometry analysis described above, a rigorous gating technique was implemented to assess surface marker expression accurately. Leukemic blasts were characterized by diminished CD45 expression and reduced side scatter. The expression of CD34 and CD38 delineated the cellular compartments of CD34-positive ALLs, and the expression of all markers was assessed within these distinct cellular compartments. ALL instances are categorized by type of ALL, gender, age demographics, and residency status. Hematological markers, including hemoglobin, platelets, white blood cells, peripheral blood blasts, and bone

marrow blasts, were analyzed in all 50 patients before and following treatment.

Statistical analysis:

Statistical analyses were conducted using NCSS 2021 (version 21.0.3, USA) and GraphPad Prism software (version 9.01, USA). This 2^−ΔΔCT approach quantified the relative abundance of each miRNA, normalizing data against SNU6 as an internal control for dissimilarities in cDNA input. In addition, the Shapiro–Wilk and the Kolmogorov–Smirnov test was utilized to detect the normal distribution of the data. The student's t-test was performed for normally distributed data, expressed as Mean±SEM; whereas, the Wilcoxon signed-rank test was employed for non-normally distributed data, expressed as median (range). The Kruskal-Wallis test was used to compare the groups. *p*-values ≤ 0.05 were considered statistically significant for all analyses. The correlations between clinical parameters and miRNA expression levels in patients were calculated by performing Spearman's rank correlation test. MedCalcR v20.215 software performed the receiver operating characteristic (ROC) analysis.

3. RESULTS

Demographics of ALL patients and healthy controls:

Comprehensive baseline characteristics, including demographic, laboratory data, and biochemical parameters, are summarized in Table 2. Overall, the comparisons among various parameters between a control group (n=20) and ALL patients (n=25) are presented. The mean age of the ALL group (15.782±2.921 years) is slightly lower than the control group (19.40±2.449 years), but the substantial differences were not recorded between the study groups (*p*-value= 0.2047). Males are more prevalent in the ALL patients with 68% compared to the healthy control group (55%), though no statistical analysis is provided for this variable. Risk factors include family history: four individuals in the ALL group reported a history of the disease, whereas none in the control group did. And also, the proportion of smokers is similar between the groups. The main parameters related to our sample study, hematological parameters, which comprise the WBC Count, were significantly elevated in the ALL group (*p*-value=0.0492), indicating leukocytosis. lymphocytes and LYM%, higher in the ALL group (*p*-value=0.0173 and *p*-value=0.0013, respectively), reflecting lymphocytic proliferation. Monocytes and MID% were increased in the ALL group (*p*-value=0.0329 and *p*-value=0.0248), suggesting a shift in immune cell populations. Granulocytes and GRA%, although the granulocyte count is not significantly different (*p*-value=0.6338), the percentage is significantly lower in the ALL group (*p*-value=0.0003), reflecting a shift in white blood cell composition. Blast cells were presented only in the ALL group, indicating leukemic transformation. RBC Count and hemoglobin (Baradaran *et al.*) were both significantly lower in the ALL group (*p*-value<0.0000), suggesting anemia. Platelet Count was markedly reduced in the ALL group (*p*-value=0.0272), consistent with bone marrow suppression. Biochemical parameters, including glucose (Lyssenko *et al.*), are elevated in the ALL group but show no significant difference. Urea is significantly higher in the ALL group (*p*-value=0.0495), possibly indicating renal involvement, whereas creatinine levels show no significant difference (*p*-value=0.596). There is no substantial difference between groups in uric acid (*p*-value=0.554). GOT and GPT are significantly elevated (*p*-value=0.0371 and *p*-value<0.0000, respectively), indicating liver involvement. Total bilirubin is slightly higher but not statistically significant (*p*-value=0.0657). LDH-P and ALP were significantly elevated in the ALL group (*p*-value<0.0000 and *p*-value=0.0236, respectively), suggesting high cell turnover. CRP (C-reactive protein) significantly increased in the ALL group (*p*-value=0.003), indicating an inflammatory response. Patients with ALL show significant hematological abnormalities, including increased WBC count, lymphocytosis, anemia, and thrombocytopenia. Biochemical markers such as LDH, liver enzymes, and CRP are also significantly altered, reflecting the systemic impact of leukemia. These findings align with the pathophysiology of ALL, characterized by bone marrow infiltration, immune dysregulation, and metabolic disturbances.

Table 2: Demographic and clinicopathological features of the ALL patients and healthy groups.

Parameters	Healthy control (n=20)	Acute lymphoblastic leukemia (n=25)	*p*-value
Age	19.40±2.449*	15.782±2.921	0.2047[ns]
Sex (M/F)	11/9 (55/45)	17/8 (68/32)	
Family history	0	4	---------
Smoking	5	4	
WBC	6.92±0.348	15.086±3.589	**0.0492***
Lymphocytes	2.515±0.204	7.309±1.719	**0.0173***
LYM%	36.415±2.126	55.472±4.625	**0.0013****
Monocytes	0.45±0.0295	2.7976±0.95	**0.0329***
MID%	7.275±0.332	11.476±1.589	**0.0248***
Granulocytes	3.955±0.247	4.979±1.894	0.6338[ns]
GRA%	56.3±2.195	33.052±5.003	**0.0003*****
Blast	39.63±2.351	42.2±6.428	**0.7823[#]**
RBC-C	5.278±0.172	3.272±0.178	**0.0000******
Hb	15.03±0.49	9.424±0.471	**0.0000******
Platelets	258.5±24.986	153.6±35.78	**0.0272***
Glu	99.33±3.219	108.62±8.148	**0.0278***
Urea	24.97±0.926	29.736±1.97	**0.0495***
Creatinine	0.6145±0.0535	0.668±0.078	0.5958[ns]
Uric acid	4.72±0.314	5.138±0.568	0.5545[ns]
GOT	29.389±7.765	53.92±17.484	**0.0371***
GPT	28.23±15.211	43.718±6.97	**0.0000******
T. Bilirubin	0.49±0.062	1.088±0.278	0.0657[ns]
LDH-P	225.21±10.518	1368.927±495.146	**0.0000******
ALP	113.18±19.471	189.333±25.263	**0.0236***
CRP	2.50±0.932	68.363±15.474	**0.0030****

*: Data are described as mean ± standard error (SEM); MID%, Mid-range absolute count; ALT (GPT): Alanine aminotransferase (glutamyl pyruvic transaminase); AST (GOT): Aspartate aminotransferase (glutamyl oxaloacetic transaminase); LDH, lactate dehydrogenase; ALP, alkaline phosphatase; CRP, C-reactive protein. Bold *p*-values indicate statistically significant differences.

Bisphenol A (BPA) concentrations in different study groups:

The box plot (Figure 1) illustrates the concentration levels of BPA (ng/mL) across three study groups: Control (2.264±0.234), new cases (7.022±1.15), and follow-up cases (9.115±2.289). The concentration of BPA was meaningfully lower in the control group compared to the latest and follow-up cases of ALL patients (*p*-value=0.0001), suggesting a possible link between BPA exposure and ALL patients. No significant difference is observed between the newly diagnosed ALL and follow-up ALL groups (*p*-value=0.9287), indicating that BPA levels remain elevated after diagnosis and treatment. The findings suggest that BPA exposure may be associated with the presence or progression of ALL, warranting further investigation into its role in leukemia development and persistence. The Mean±SEM of BPA concentration levels in healthy controls was 2.264±0.234, significantly lower when compared to new cases (7.022±1.15) (*p*-value=0.0001), and follow-up cases (9.115±2.289) (*p*-value=0.0001). At the same time, this was not significant between new and follow-up cases of ALL (*p*-value=0.9998).

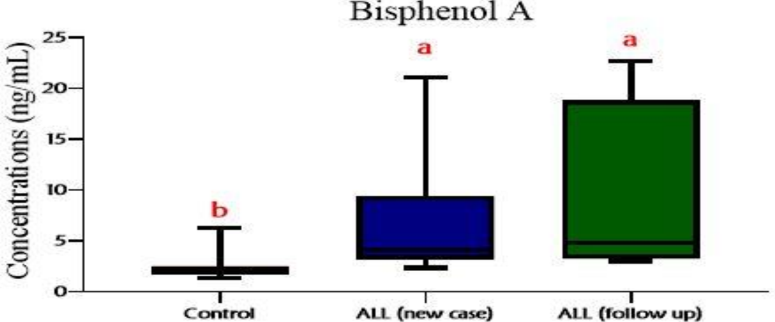
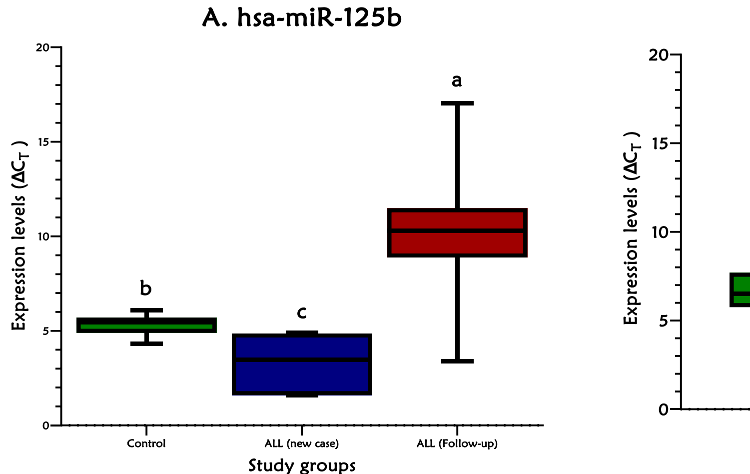


Figure 1: Concentration levels of Bisphenol A in ALL patients and healthy groups.

Expression of the miRNAs:

Figure 2 illustrates that the qRT-PCR data indicated a substantial upregulation in the levels of hsa-miR-125b expression (Δ_{CT} value) among individuals newly diagnosed with ALL compared to healthy controls (normal controls; 5.325 ± 0.162, new cases 3.319±0.309, *p*-value=0.002). Conversely, the expression level of hsa-miR-125b in follow-up cases was downregulated and significantly different from that of healthy controls (Δ_{CT} value=9.299±0.569). Similarly, the Δ_{CT} value of hsa-miR-574 was considerably reduced in patients compared to controls (normal controls: 6.753 ± 0.271; new cases: 10.376 ± 0.476; *p*-value = 0.0001). Conversely, the expression (Δ_{CT} value) of hsa-miR-574 in follow-up cases was significantly upregulated compared to samples from new cases, returning to levels observed in normal controls (ALL follow-up patients, Δ_{CT} value=5.359 ± 0.34). As mentioned earlier, the expression data were standardized to the internal control (SNRU6) before analysis. Data are presented as mean values with corresponding standard errors (SEM).



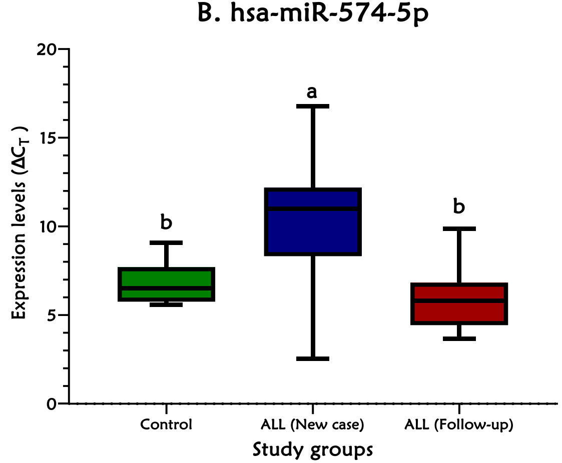


Figure 2: Expression levels (Δ_{CT} value) of miRNAs in ALL patients and the healthy group. (A). hsa-miR-125b, and (B). hsa-miR-574. The results represent the mean values of the healthy group (*n* = 20) and ALL patients (new cases, *n* = 25, and follow-up, *n* = 25). Distinct letters denote a substantially difference among the groups.

The expression levels of both miRNAs are quantified as relative expression (fold change $2^{\wedge-\Delta\Delta CT}$). The hsa-miR-125b expression levels were meaningfully upregulated in the new cases of the ALL-patients group compared to healthy controls, indicating a 6.517-fold increase. Conversely, hsa-miR-574 exhibited a down-regulation of 7.496-fold in new cases of ALL patients. In follow-up cases, hsa-miR-125b expression was significantly downregulated compared to the normal control group, reflecting a 4.83-fold decrease. Additionally, hsa-miR-574 expression in follow-up cases of ALL patients was upregulated, showing a 3.195-fold increase (Table 3). Based on the ROC curve, the expression of hsa-miR-125b (new cases) with AUC = 0.917 (*p*-value=0.001, 95%CI: 0.797-0.984, the hsa-miR-125b (follow-up cases) with AUC = 0.888 (*p*-value=0.001, 95%CI: 0.743-0.967), the hsa-miR-574 (new cases) with AUC = 0.953 (*p*-value=0.001, 95%CI: 0.829-0.996) and hsa-miR-574 (follow-up cases) with AUC = 0.640 (*p*-value=0.0129, 95%CI: 0.466-0.791) were correlated with the ALL cases (Figure 3).

Table 3: Relative expression ($2^{\wedge-\Delta\Delta CT}$) of different miRNAs in new and follow-up cases of ALL patients.

miRNA	ALL cases	Relative expression	*p*-value	Overall expression
hsa-miR-125b	New	6.517±0.011	0.0001*	Up regulation
	Follow-up	-4.83±1.067	0.0001*	Down regulation
hsa-miR-574	New	-7.496±4.107	0.0001*	Down regulation
	Follow-up	3.195±0.132	0.0001*	Up regulation

*: Fold change is obtainable as Mean±SEM. **p*-value ≤ 0.05 is measured as significant different between study groups. The total expression displays changes in expression between the ALL patients and the healthy groups.

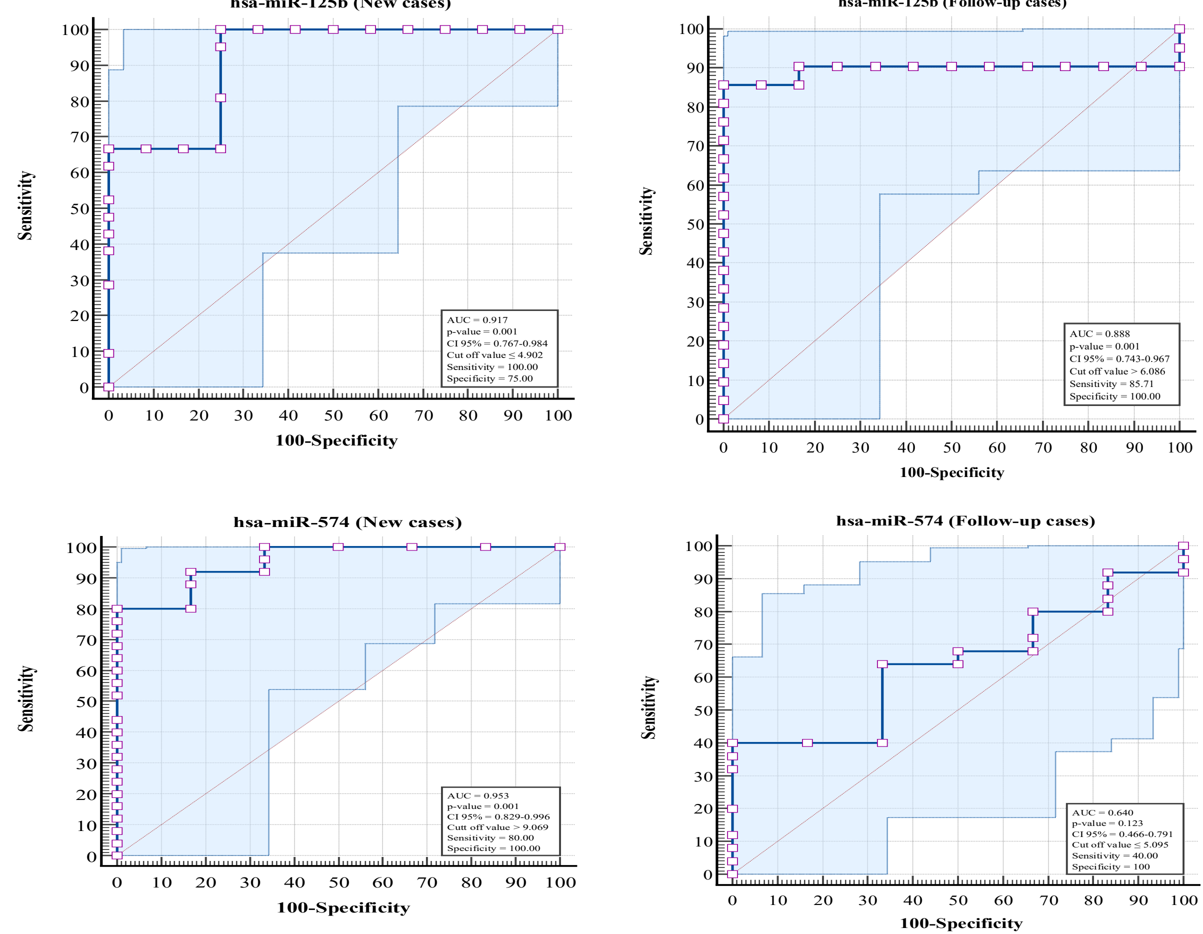


Figure 3: The ROC curve analysis: (A) hsa-miR-125b (new cases), (B) hsa-miR-125b (follow-up cases), (C) hsa-miR-574 (new cases), and (D) hsa-miR-574 (follow-up cases). The consequences are revealed as the area under the ROC curve (Dumortier *et al.*) for the sensitivity and specificity of both miRNAs.

A high sensitivity in a ROC curve analysis indicates that the diagnostic test or predictive model can correctly identify true positive cases, i.e., it effectively detects individuals with the condition of interest. This minimizes the likelihood of false negatives, particularly in clinical scenarios where missing a diagnosis could have serious consequences. Similarly, a high specificity reflects the model's accuracy in correctly identifying true negative cases, thereby reducing the occurrence of false positives. This is especially critical in contexts where unnecessary treatment or anxiety resulting from incorrect

positive results must be avoided. When both sensitivity and specificity are high, the corresponding ROC curve rises sharply toward the top-left corner of the graph. This indicates excellent discriminative power, where the model can accurately distinguish between positive and negative classes. The AUC typically approaches 1.0 in such cases, suggesting outstanding diagnostic performance. High sensitivity and specificity imply that the test achieves a balanced and accurate classification, minimizing diagnostic errors. This enhances its clinical or practical applicability, supporting its use as a reliable tool for decision-making.

Correlation of miRNA expression levels with CD markers:

Furthermore, depending on the unique biomolecular systems associated with healthy and malignant conditions, we hypothesized that miRNA expression relationships with CD markers could benefit the early onset of ALL. In this study, a correlation among the expression levels of miRNAs and CD markers was found, and in certain instances, the correlations were positive. In some instances, miRNA expression was found to have an inverse correlation with CD markers. For instance, as seen in Figure 4, hsa-miR-125b in new cases was found to have a positive connection with the expression of HLA-DR ($r = 0.995$, as a high positive correlation among miRNAs and CD markers). Additionally, hsa-miR-125b in follow-up cases was found to have a high positive connection with the expression of CD11b and CD11c ($r = 0.958$). On the contrary, the expression of hsa-miR-574 in the new cases, was found to have a high significant association with the expression of CD13 ($r = 0.965$, as a high correlation among miRNAs and CD markers), even though, the expression of hsa-miR-574 in the follow-up cases, was found to have a high association with the expression of CD79a ($r = 0.999$, as a high correlation among miRNAs and CD markers).

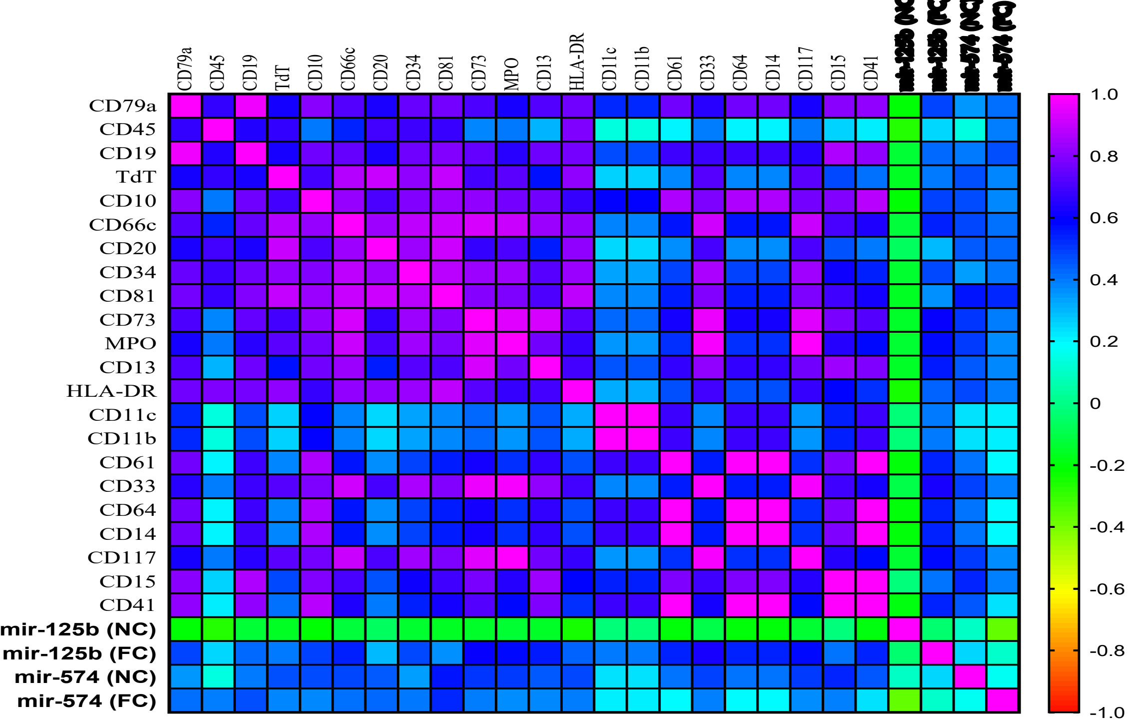


Figure 4: Valuation of miRNA expression and its correlation with CD markers.

Association of miRNA expression levels with bisphenol A concentrations:

Bisphenol A positively correlated with expression levels of hsa-miRNAs in ALL patients (Figure 5). When the level of bisphenol A increased in new cases of ALL patients, the expression level of hsa-miR-125b upregulates, and a moderate positive correlation with hsa-miR-574 ($r = 0.608$) was recorded, with weak to negative correlations with hsa-miR-125b ($r = –0.204$). So, the bisphenol A in new cases appears to upregulate hsa-miR-574 notably, while showing neutral to mild downregulation of hsa-miR-125b variants. However, in the follow-up cases, the bisphenol A concentrations have a moderate positive correlation with hsa-miR-574 ($r = 0.437$), very weak or negative with hsa-hsa-miR-574 variants (ranging from ~0.03 to – 0.085). Bisphenol A in follow-up cases tends to correlate more with hsa-miR-125b, and shows minimal or no correlation with hsa-miR-574 variants.

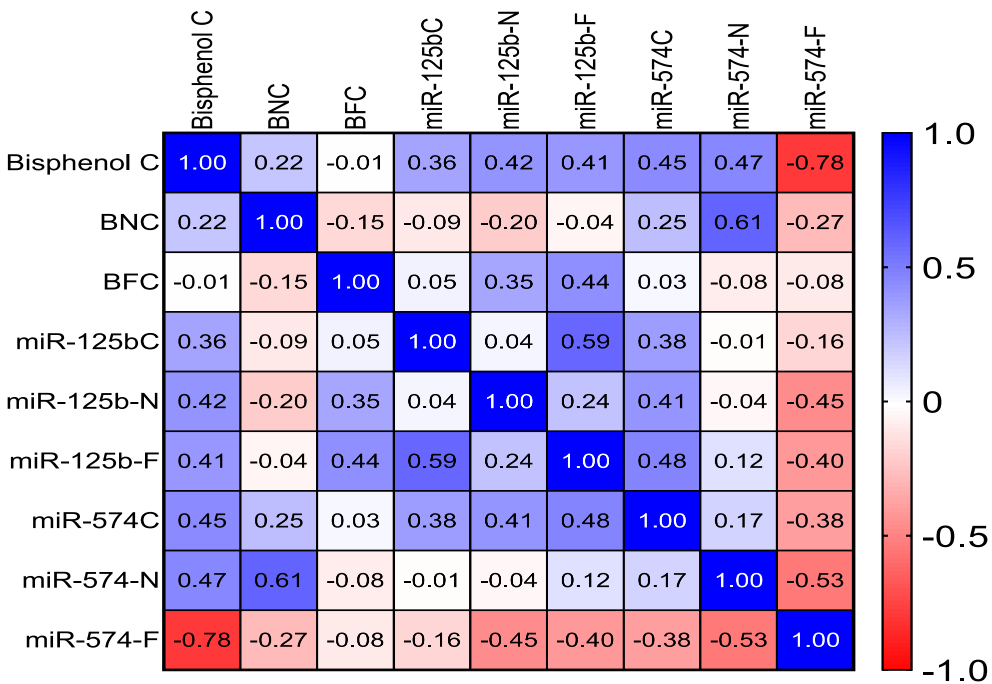


Figure 5: Correlation heatmap between the bisphenol A levels with the expression levels of hsa-miRNAs in ALL patients.

4. DISCUSSION

Preliminary clinical and experimental data:

The median ages of the ALL patients and the control groups were 8.4 and 10.2 years, respectively. The prevalence of ALL patients was male (55.0%) and detected with precursor B-ALL. In the comprehensive ALL patient group, within the precursor B-ALL subgroup, the median and mean percentages of bone marrow blasts at diagnosis were 88.21% and 86.55%, respectively. Corresponding to our results, the research conducted by Piatopoulou *et al*. (2017) revealed that the median age of the ALL and healthy groups was 5.0 and 4.0 years, respectively. The predominance of the ALL group was male (58.4%), diagnosed with precursor B-ALL (83.2% CD10+ and 4.8% CD10−), and displaying white blood cell counts < 50,000 cells per µL (81.6%).

Evaluation of Bisphenol A (BPA) concentrations:

The current study lacks data regarding BPA concentrations in persons diagnosed with ALL compared to healthy individuals. Nevertheless, research has quantified BPA concentrations in the general populace, providing a reference point for comparison (Colorado-Yohar *et al*., 2021). A systematic review and meta-analysis of 15 investigations, including 28353 participants, revealed that BPA was identified in more than 90% of persons. The aggregated estimations for BPA concentrations were as follows: in creatinine-adjusted urine, BPA: 1.76 µg/g (95% Prediction Interval [PI]: 0.79–2.73), and in unadjusted urinary BPA: 1.91 µg/L (95% PI: 0–3.97). Additionally, serum BPA levels were measured at 1.75 µg/L (95% prediction interval: 0–10.58).

Expression of the studied miRNAs:

The hsa-miR-125b expression levels in ALL patients have been considered because of its possible role in disease progression and cure response. Current investigations have provided insights into its expression patterns and clinical implications in B-ALL and T-ALL. In pediatric B-ALL patients, hsa-miR-125b levels are meaningfully downregulated in new cases compared to the control group. Following induction therapy with the Berlin–Frankfurt–Münster (BFM) procedure, hsa-miR-125b expression increases. However, a higher ratio of hsa-miR-125b expression on day 33 post-treatment relative to diagnosis is associated with unfavorable prognostic features, including: T-lineage ALL, elevated white blood cell counts (≥50,000/µL), age extremes (<1 or ≥10 years), intermediate/high-risk classification (Piatopoulou *et al*., 2017). Patients with this expression pattern also show an increased risk of short-term recurrence and diminished overall survival. Integrating hsa-miR-125b expression data with recognized prognostic indicators improves resistance to BFM treatment prediction. In T-ALL, hsa-miR-125b influences leukemic cell differentiation and metabolism. The hsa-miR-125b is overexpressed in undifferentiated CD4-negative leukemic T cells. The overexpression facilitates a more undifferentiated state and increases glucose uptake and oxygen consumption by directly influencing the A20 (TNFAIP3) gene. Restoration of A20 expression mitigates these effects, indicating that hsa-mir-125b performs a function in T-ALL formation via metabolic reprogramming and differentiation inhibition (Liu *et al*., 2016). In TEL-AML1-positive B-ALL, coexpression of hsa-miR-125b with hsa-miR-99a or hsa-miR-100 significantly induces resistance to vincristine, a key chemotherapeutic agent. While overexpression of hsa-miR-125b alone does not markedly affect cell survival, its combination with hsa-miR-99a or hsa-miR-100 results in a substantial increase in cell viability post-vincristine treatment. This synergy suggests that hsa-miR-125b, in concert with other miRNAs, contributes to chemotherapy resistance in specific ALL subtypes (Moqadam *et al*., 2012).

In recent decades, significant advancements in treating ALL have greatly improved remission rates and overall survival. Despite this progress, clinical challenges remain—some patients are subjected to overtreatment or face poor outcomes even when their initial clinical indicators appear satisfactory (Pui *et al*., 2012). This underscores the need for new prognostic markers to more accurately track treatment response and predict patient outcomes. Recently, miRNAs have gained attention as promising biomarkers across various cancers, including ALL, with growing evidence supporting their role in leukemia development and their impact on disease progression and patient survival (de Oliveira *et al*., 2012).

The miRNA dysregulation is common in B-ALL and T-ALL, performing as either oncomiRs or tumor suppressor miRNAs (Grobbelaar & Ford, 2019). A unique miRNA expression profile, predominantly featuring "hsa-miR-92a, hsa-miR-100, hsa-miR-125b, hsa-miR-128a, hsa-miR-181b, and hsa-miR-196b", was discerned once comparison with B-ALL lymphoblasts to normal CD34+ cells (Schotte *et al*., 2012). In cases with ALL patients, the utmost recurrently altered miRNAs encompass the hsa-miR-181 cluster, often noted for its upregulation and recognized as a vital oncomiR in pediatric ALL; hsa-miR-155, which enhances the clonal proliferation of pre-B-cells and is upregulated across multiple pediatric ALL subtypes; and hsa-miR-128b, which aids in differentiation from AML cases and is down-regulated in ALL linked to the MLL-AF4 translocation (Anelli *et al*., 2021). In ALL, the downregulation of hsa-miR-708 and let-7b is commonly reported, possibly due to hypermethylation in DNA generated by the MLL fusion protein. Further, often uncontrolled miRNAs in ALL encompass "hsa-miR-100, hsa-miR-125b, hsa-miR-99a, and hsa-miR-126", which are upregulated in ETV6-RUNX1 patients (Szczepanek, 2020). In cases of BCR-ABL1-positive ALL, the hsa-miR-125b expression level is down-regulated at diagnosis but is considerably overexpressed around one month later (Grobbelaar & Ford, 2019). In patients with ALL cases, the downregulation of hsa-miR-708 is recurrently reported, possibly due to DNA hypermethylation generated by the MLL fusion protein.

In contrast to our findings, in the investigations of Piatopoulou *et al*. (2017), the study of expression levels indicated a substantial reduction in hsa-miR-125b in children with ALL compared to the healthy control group (*p*-value=0.004). The capacity of hsa-miR-125b to differentiate between ALL patients and healthy subjects was corroborated by "univariate logistic regression" (OR: 0.477; 95% CI: 0.288–0.790; *p*-value=0.004) and ROC curve analysis (area under the curve [AUC]: 0.628; 95% CI: 0.548–0.707; *p*-value=0.004). Moreover, multivariate logistic regression, adjusted for age and gender, validated that this discriminatory ability was independent of these variables (OR: 0.507; 95% CI: 0.305–0.842; *p*-value=0.009). Kyriakidis *et al*. (2022) analyzed the works on contrastingly expressed miRNAs across juvenile ALL subtypes and observed substantial variation among investigations, especially in subgroup classification within ALL. A study of published data suggested the contribution of several miRNAs in the pathogenesis of ALL, as documented by two or more studies. Childhood ALL is specifically correlated with the elevation of "miR-16, miR-19b, miR-92a, miR-130b, miR-146a, miR-181a, miR-181b, and miR-221", alongside the down-regulation of "miR-29a, miR-145, and miR-574" when compared to controls. Two studies have identified significantly reduced expression levels of miR-145 and miR-574 in children with T-ALL compared to healthy individuals (Dawidowska *et al*., 2019; Duyu *et al*., 2014). Declined expression levels of miR-574 appear to successfully distinguish T-ALL from B-ALL, while instances with TLX1 deregulation appear to be excluded from this trend (Almeida *et al*., 2019).

The significantly increased expression levels of miR-574 turned out to be detected in the BCP-ALL, KMT2A-r, and TCF3-PBX1 subtypes when compared with other cases of B-ALL (Alsuwaidi *et al*., 2021). Current evidence on how miRNAs are expressed differently in children with ALL compared to healthy controls highlights several critical observations. (i) Because study designs vary so much in the published literature, it is impossible to do an unbiased meta-analysis. (ii) A strongly associated between the development of pediatric ALL and the significant elevation of the "miR-128, miR-130, miR-155, miR-181 families, miR-210, miR-222, miR-363, and miR-708", as well as the continuous down-regulation of "miR-143 and miR-148a". Other miRNAs that are assumed to perform a function in the development of ALL are "miR-24, miR-99a, miR-100, miR-125b, miR-145, miR-146a, miR-182, miR-196b, miR-199b, miR-223, and miR-335". (iii) It is imperative to note that the elevation of "miR-130b, and miR-181", together with the down-regulation of miR-145 and miR-574, are possible biomarkers for childhood T-ALL (Kyriakidis *et al*., 2022). hsa-miR-574, whose diminished expression appears to facilitate the proliferation and survival of leukemic cells by pointing the IL6/JAK/STAT3 pathway; (iv) hsa-miR-618, whose reduced expression has been associated with childhood ALL and is also believed to contribute to lymphoma development through p53, STAT3, HDAC3 (histone deacetylase 3), CUL4A (cullin 4A), and FKBP3 (Liang *et al*., 2021).

Correlation of miRNA expression levels with CD markers:

Based on observing distinct biomolecular networks associated with both healthy and cancerous conditions, we proposed that analyzing the relationship between miRNA expression and CD markers could aid in the early detection of ALL. Our study revealed that miRNAs and CD markers often show significant positive and inverse correlations. For instance, in newly diagnosed cases, hsa-miR-125b showed a strong positive correlation with HLA-DR expression (r=0.995). In follow-up cases, this same miRNA also exhibited high positive correlations with CD11b and CD11c (r=0.958). Similarly, hsa-miR-574 strongly correlated with CD13 in new cases (r=0.965) and CD79a in follow-up cases (r=0.999).

In T-ALL, the expression levels of hsa-miR-125b are notably elevated in undifferentiated T-cells lacking CD4 expression. Over-expression of hsa-miR-125b increases the population of CD4-negative T cells, while its inhibition leads to a decrease in this population. This proposes that hsa-miR-125b contributes to maintaining an undifferentiated state in T cells by repressing CD4 expression. Mechanistically, hsa-miR-125b directly targets A20 (TNFAIP3), a negative regulator of the NF-κB mechanism, leading to enhanced glucose metabolism and proliferation in T-ALL cells. In childhood ALL, hsa-miR-125b expression levels at diagnosis have been linked to specific immunophenotypic features: patients with T-lineage ALL, characterized by markers such as CD3, CD7, and CD5, exhibited significantly lower hsa-miR-125b levels at diagnosis compared to those with B-lineage ALL (e.g., CD19, CD10, CD20). This suggests an association between reduced hsa-miR-125b expression and T-cell immunophenotype. Lower hsa-miR-125b levels at diagnosis correlated with unfavorable prognostic features, including elevated white blood cell counts (≥50,000/μL), age extremes (<1 or ≥10 years), and intermediate/high-risk classifications. Furthermore, an increased expression ratio of hsa-mir-125b on day 33 post-treatment relative to diagnosis was interrelated with a higher risk of short-term relapse and poorer overall survival (Liu *et al*., 2016).

A study by Piatopoulou *et al*. (2017) analyzed the expression levels of hsa-miR-125b in T-ALL patients, who typically express CD3, CD5, and CD7, and found that these levels were significantly decreased compared to B-cell precursor ALL patients, who express markers like CD10, CD19, and CD22. This differential expression suggests that hsa-miR-125b might performance a role in lineage specification or reflect developmental differences between T and B lineage leukemia. Patients with lower hsa-miR-125b levels often showed higher white blood cell counts and were categorized into higher-risk groups.

Correlation of bisphenol A concentrations with miRNA expression levels:

Bisphenol A (BPA) is widely used in industry, resulting in frequent exposure to the general population (Rezg *et al*., 2014). BPA is applied in many industries, thus people are exposed to it repeatedly (Rasdi *et al*., 2020). Investigations revealed a

momentous level of urinary BPA, which was evaluated in BPA-related industrial workers and children (Hartle *et al*., 2016). BPA has also been found in human serum at levels as high as 4.4 ng/mL and 11.2 ng/mL (Rasdi *et al*., 2020). The relationship between BPA, ALL, and the expression of hsa-miR-125b and hsa-miR-574 is a developing area of research. While direct studies linking BPA exposure to alterations in hsa-miR-125b and hsa-miR-574 expression in ALL are limited, existing literature provides insights into their roles in leukemia pathogenesis.

Bisphenol A (BPA) levels positively correlated with certain miRNAs' expression in ALL patients (Figure 5). In newly diagnosed ALL cases, increased BPA levels were associated with higher expression of hsa-miR-125b and a moderate positive correlation with hsa-miR-574 (r=0.608), while a weak negative association was detected with hsa-miR-125b (r=-0.204). This suggests that BPA may notably upregulate hsa-miR-574, while exerting little to no consistent effect—or a slight downregulatory effect—on hsa-miR-125b variants. In follow-up cases, BPA levels showed a moderate positive correlation with hsa-miR-574 (r=0.437), but correlations with hsa-miR-574 variants ranged from very weak to slightly negative (r=0.03 to -0.085). Conversely, BPA was more consistently correlated with hsa-miR-125b in these follow-up patients, showing minimal or negligible association with hsa-miR-574 variants.

Bisphenol A (BPA), a known endocrine-disrupting chemical, has been implicated in leukemia's pathogenesis, particularly ALL, through its influence on miRNA expression. In ALL patients, elevated BPA levels have been related to altered expression of specific miRNAs, including hsa-miR-125b and hsa-miR-574. These miRNAs are recognized to play roles in cell proliferation, apoptosis, and immune regulation, processes critical in leukemia development. In newly diagnosed ALL cases, BPA showed a moderate positive correlation with hsa-miR-574 and a variable (weak or negative) correlation with hsa-miR-125b. This suggests BPA may upregulate certain oncogenic miRNAs or disrupt normal miRNA regulation, contributing to leukemogenesis. In follow-up patients, the correlation patterns shifted, indicating a potential change in BPA's biological impact throughout treatment or disease progression.

The up-regulation of hsa-miR-125b seemed to be revealed to induce leukemia in murine models, leading to B-cell and T-cell ALL, as well as myeloproliferative neoplasms (Bousquet *et al*., 2010). hsa-miR-125b contributes to leukemogenesis by targeting multiple genes involved in cell differentiation and apoptosis. For instance, it represses the expression of IRF4, a transcription factor crucial for B-cell development, thereby promoting B-cell leukemias (So *et al*., 2014). In T-cell ALL, hsa-miR-125b targets A20 (TNFAIP3), leading to increased glucose uptake and oxygen consumption, which supports leukemic cell proliferation. The function of hsa-miR-574 in leukemia, particularly ALL, is less well-characterized than miR-125b. Some studies suggest that hsa-miR-574 may function as a tumor suppressor in certain cancers, but its specific involvement in ALL requires further investigation. BPA, an environmental endocrine disruptor, has been stated to influence the expression of various miRNAs, potentially affecting cancer development. Exposure to BPA has been associated with altered expression of several miRNAs involved in cell proliferation and apoptosis. However, in the context of ALL, direct evidence linking BPA exposure to changes in hsa-miR-125b or hsa-miR-574 expression is currently lacking (Rezg *et al*., 2014).

The hsa-miR-574 has been implicated in various cancers, often functioning as a tumor suppressor. In chronic myeloid leukemia (CML), hsa-miR-574-3p is down-regulated in patient peripheral blood samples. Overexpression of hsa-miR-574 in CML cell lines inhibits proliferation and induces apoptosis by directly targeting IL-6, thereby suppressing the IL-6/JAK/STAT3 signaling pathway (Yang *et al*., 2018). Regarding ALL, a systematic review and meta-analysis identified differential expression of several miRNAs in childhood T-cell ALL (T-ALL) compared to controls. Notably, hsa-miR-574 was among the microRNAs significantly downregulated in T-ALL samples (Kyriakidis *et al*., 2022). Previous studies have shown that estradiol can regulate elements of the miRNA processing machinery (Nothnick *et al*., 2010). The focus was on evaluating the expression of enzymes involved in miRNA processing (Drosha, Dgcr8, Dicer) and Ago2, a crucial part of the RNA-induced silencing complex (RISC) (Mak *et al*., 2023). The cell lines expressed Drosha, Dgcr8, Dicer, and Ago2, indicating they contain the necessary machinery for miRNA production. In mHypoE-46 cells, BPA reduced the mRNA levels of Drosha and Dgcr8, while Dicer and Ago2 remained unchanged. In mHypoE-41 cells, only Drosha expression was significantly reduced by BPA. However, in mHypoA-59 cells, BPA did not affect Drosha or Dgcr8, but led to decreased expression of Dicer and Ago2—an effect not seen in the other two lines. Importantly, BPA did not change the expression of these miRNA machinery genes in primary hypothalamic neuron cultures. These findings demonstrate that BPA affects components of the miRNA processing pathway in a cell-type-specific manner, reflecting its varied effects on Npy-expressing neuronal subtypes (Mak *et al*., 2023).

CONCLUSIONS

Overall, these findings suggest that BPA exposure may influence leukemia progression by modulating miRNA expression profiles, offering potential biomarkers for environmental risk and molecular mechanisms of disease.

Acknowledgements:

The authors would like to express their sincere gratitude to Salahaddin University-Erbil for providing the necessary facilities and support for this research. We are especially thankful to Nanakali Hospital for Cancer and Blood Diseases, whose valuable insights, guidance, and encouragement greatly contributed to completing this work.

Ethical Statement:

This study design was approved by the ethical code SUE2025HREC-25 in the Research Center at Salahaddin University-Erbil. Also, consent forms were completed for all participants.

Author Contribution:

S. A. Y: writing, original draft preparation, formal analysis, reviewing, and editing. R. M. H: supervision, resources and data curation, conceptualization and visualization, statistics and validation, software.

Conflict of interest:

None declared.

Declaration statement:

The authors state that all information in this article is accurate to the best of our knowledge, and we take full responsibility for its correctness.

Funding:

This research did not receive any specific funding from public, commercial, or non-profit organizations.

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