



The study of Relationship between Dysregulation of *RP11-446E9*, *HOTAIR*, and *P21* Long non-coding RNA and Acute Lymphocytic Leukemia Patients

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ABSTRACT

Acute lymphoblastic leukemia is a kind of hematological cancer resulting from the aberrant proliferation of immature lymphoid cells. Recently discovered lncRNAs longer than 200 nt interact with various cellular processes, including pluripotency and oncogenesis. Studies show that lncRNA expression profiles in ALL can serve as early diagnostic and prognostic indicators. The purpose of this work is to assess the lncRNA expression profile of three lncRNAs, including lncRNA *RP11-446E9*, lncRNA *HOTAIR* and lncRNA *P21*, in newly diagnosed ALL patients and controls, and to calculate the relationship between some blood parameters like, White blood cell count, hemoglobin and blood platelets and these three lncRNA expression patterns, as well as to determine whether an association between the expression of lncRNAs *RP11-446E9*, *HOTAIR*, *P21* expression and cluster of differentiation are occur or not. We obtained 30 patient blood samples for this investigation, with an equal number from healthy donors. We extracted total RNA from these samples and synthesized cDNA. We then assessed lncRNA expression levels by real-time PCR, with P-values <0.05 considered statistically significant. Our results showed that ALL samples had significantly higher expression levels of the lncRNAs *RP11-446E9*, *HOTAIR*, and *P21* than healthy samples ($P = 0.0218$, $p < 0.0001$, $p = 0.0252$). Moreover, the relationships between these lncRNAs and Cluster of Differentiation markers and blood parameters varied. Dysregulation of lncRNAs in ALL samples offers promising potential for early diagnosis, prognosis, and treatment management.

Keywords: *ALL, RP11-446E9, HOTAIR, P21, Blood parameter.*

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دراسة العلاقة بين خلل تنظيم *RP11-446E9* و *HOTAIR* و *P21 RNA* الطويل غير المشفر

ومرضى سرطان الدم الليمفاوي الحاد

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الملخص

سرطان الدم الليمفاوي الحاد هو نوع من أنواع سرطان الدم الناتج عن الانتشار غير الطبيعي للخلايا الليمفاوية غير الناضجة. تتفاعل جزيئات lncRNAs المُكتشفة حديثاً، والتي يزيد طولها عن 200 نكليوتيد، مع عمليات خلوية مختلفة، بما في ذلك تعدد القدرات وتكوين الأورام. تكشف الدراسات أن ملفات تعريف التعبير لـ lncRNA في سرطان الدم الليمفاوي الحاد يمكن أن تكون بمثابة مؤشرات تشخيصية وتنبؤية مبكرة. الغرض من هذا العمل هو تقييم ملف تعبير lncRNA لثلاثة lncRNAs بما في ذلك *lncRNA RP11-446E9*، و *lncRNA HOTAIR* و *lncRNA P21* في المرضى الذين تم تشخيصهم حديثاً بسرطان الدم الليمفاوي الحاد ومجموعة التحكم، وحساب العلاقة بين بعض معايير الدم مثل عدد كريات الدم البيضاء، والهيموغلوبين، والصفائح الدموية مع نمط تعبير lncRNA الثلاثة هذا، بالإضافة إلى معرفة ما إذا كان الارتباط بين تعبير *lncRNAs RP11-446E9* و *HOTAIR* و *P21* ومجموعة التمايز يحدث أم لا. تم الحصول على ثلاثين عينة دم من مرضى لهذا البحث، وعدد مماثل من متبرعين أصحاء. من هذه العينات، استُخلص الحمض النووي الريبسي الكلي، وأُنتج الحمض النووي الريبسي التكميلي. ثم قُيِّمت مستويات التعبير عن الحمض النووي الريبسي غير المتمائل (lncRNAs) باستخدام تفاعل البوليميراز المتسلسل في الوقت الفعلي، حيث تُعتبر قيمة الاحتمال (P) الأقل من 0.05 ذات دلالة إحصائية. وفقاً لنتائجنا، أظهرت عينات سرطان الدم الليمفاوي الحاد مستويات أعلى بكثير من التعبير الجيني لـ *lncRNAs RP11-446E9* و *HOTAIR* و *P21* مقارنةً بالعينات السليمة ($P = 0.0218$ ، $p > 0.0001$ ، $p = 0.0252$). علاوة على ذلك، تباينت العلاقة بين هذه الـ lncRNAs وعلامات التمايز العنقودية ومعايير الدم. يوفر اختلال تنظيم الـ lncRNAs في عينات سرطان الدم الليمفاوي الحاد إمكانيات واعدة للتشخيص المبكر للمرض، وتوقع مساره، وإدارة علاجه.

1. INTRODUCTION

Leukemia is characterized by the uncontrolled growth of white blood cells in the blood plus bone marrow. There are four kinds of leukemia: Chronic Lymphocytic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL), Chronic Myeloid Leukemia (CML), and Acute Myeloid Leukemia (AML)⁽¹⁾. It is a genetic disorder in the blood cells. It is characterized by low hemoglobin and a lower red blood cell count than the normal range.⁽²⁾

Bone marrow, peripheral blood, and extramedullary areas are invaded by aberrant, immature lymphoid progenitors of B-cell or T-cell origin, which clonally proliferate to form ALL (Figure 1). This type of cancer of the bone marrow and blood damages platelets, red blood cells and WBCs⁽³⁾.

Children are the main victims of ALL, a blood cancer that can also strike adults. T cell lineages (10–15%) and B cell lineages (85%) make up ALL.⁽⁴⁾

These immature, defective lymphocytes pose a considerable threat to the entire immune system. This abnormality also affects the bone marrow's ability to create platelets and red blood cells. Additionally, altered erythrocytes in the bloodstream may cause major health consequences for other important organs.⁽⁵⁾ This illness increases the risk of anemia, recurrent infections, and easy bruising and bleeding by interfering with the bone marrow's normal production of RBCs, WBCs, and platelets. Additionally, blast cells can go from the

Therefore, this study aims to analyze the expression level of three lncRNAs lncRNA *RP11-446E9*, lncRNA *HOTAIR*, lncRNA *P21* in newly diagnosis in ALL patient and control, their expression level was in relation with some blood parameter like WBC, Hb, and blood platelets, as well as to find out whether association between lncRNA *RP11-446E9*, lncRNA *HOTAIR*, and lncRNA *P21* expression and cluster of differentiation are present or not.

2. MATERIALS AND METHODS

2.1 Blood Sampling:

We obtained blood samples for this study from two groups: healthy donors (matched for age and gender) and patients newly diagnosed with ALL. We collected these samples at Nanakuli Hospital for Blood Diseases and Cancer in Erbil, Iraq. This study was conducted between 11th August 2024 and 7th January 2025, following written informed consent from all participants. We considered all required ethical considerations during sample collection. We collected intravenous blood samples from each subject in both groups. We collected peripheral blood samples in EDTA tubes containing an anticoagulant: one tube for Complete Blood Count (CBC) and flow cytometry and another for Long noncoding RNA analysis. We then sent the samples to the laboratory, where they were stored at -80 °C. We collected 60 samples: 30 from patients with ALL and 30 controls. In the ALL-patient group, the mean ($\pm$ SE) age was 17.89 ($\pm$ 3.087). The group included 19 (63.33%) males and 11 (36.66%) females, aged 1 to 54 years. All patients underwent a comprehensive examination, including morphological and flow cytometric studies, a detailed review of the patient's medical history, and a thorough clinical evaluation. We based the diagnosis on the French-American-British (FAB) classification and World Health Organization (WHO) standards.

We included patients who had never had an acute viral infection, an autoimmune disease, or other cancers and their associated treatments. The control group consisted of samples obtained from healthy subjects.

Ethical Approval: The Human Ethics Committee of Salahaddin University-Erbil's College of Science approved all experimental protocols (Approval Ref No: 4s/37, dated 2025/2/25).

2.2 RNA Extraction from Blood Samples and cDNA Synthesis

Using the QIAamp RNA Blood Mini Kit (QIAGEN Co., Germany), total RNA was extracted from blood samples according to the manufacturer's instructions. The concentration of extracted RNA was measured by measuring the 260/280 nm wavelength ratio with a NanoDrop spectrometer (Biometrics, OneDrop TOUCH Pro/Lite Micro-Volume Spectrophotometer, Wilmington, USA). The isolated RNA was converted to cDNA using the UltraScript® Reverse Transcriptase and cDNA Synthesis kit (PCR BIOSYSTEM Co, UK), following the manufacturer's instructions.

2.3 RT-qPCR

We examined the lncRNAs *RP11-446E9*, *HOTAIR*, and *P21* for alterations in gene expression in both normal and ALL samples using RT-qPCR. RT-qPCR was carried out with the following monitoring primers and GAPDH as the internal reference to identify variations in each gene's expression in ALL and control samples (Table 1):

Table 1: List of primers used in the present study.

Primer name	Primer Sequence '5-----3'	References
<i>RP11-446E9</i> forward	5'- CCAGAGCAGAGCATCAAGAG-3'	(19)
<i>RP11-446E9</i> reverse	5'-GAAGCACTTTGGGAAATGGA -3'	(19)
<i>HOTAIR</i> forward	5'-GCACCGCTTTTCTAACTGGC-3'	(20)
<i>HOTAIR</i> reverse	5'-CAGGGTCCCACTGCATAATCA-3'	(20)
<i>P21</i> forward	5'-AGGACCAGAATAACCCGAGC-3'	(20)
<i>P21</i> reverse	5'-CTGGGGTCCAGGATGCATAG-3'	(20)
GAPDH gene: forward	5'-GCCGTCTAGAAAAACCTGCC-3'	(19)
GAPDH gene: reverse	5'-ACCACCTGGTGCTCAGTGTA-3'	(19)

Following the manufacturer's instructions, RT-qPCR was performed using the SYBR Green method to assess expression changes in ALL and healthy samples (2X qPCRBIO SyGreen Blue mix, Co. PCR BIOSYSTEM, UK). Each PCR tube included a total of 20 μ l of primer sequences used in

this investigation for the RT-PCR procedure. The components were 12 μ L of PCR mix (10 μ L 2X qPCR BIO SyGreen Blue mix, 1 μ L forward primer, 1 μ L reverse primer), 5.5 μ L of PCR-grade dH₂O, and 2.5 μ L of cDNA template, combined to a final volume of 20 μ L.

qRT-PCR amplification was run with cycling conditions optimized (Table 2). To determine relative gene expression, expression rates were also examined using the 2-($\Delta\Delta$ CT) technique.

Table 2: Conduction temperature of Long non-coding RNA and GAPDH housekeeping expression protocol.

Temperature	Time	Cycle
95 °C	2 min	Initial denaturation
95 °C	5 sec	Denaturation
60°C	30 sec	
65°C	5 sec	Annealing
95 °C	5 sec	Elongation

2.4 Statistical Analyses

GraphPad Prism statistical software (version 9.01) was used to conduct statistical tests. To assess whether the data were normally distributed, we also used the D'Agostino-Pearson test, the Shapiro-Wilk normality test, and the Kolmogorov-Smirnov test. A t-test for normally distributed data, shown as means $\pm$ SEM, was used to compare blood parameters between patients and controls and to determine Δ CT for each lncRNA in patients and controls. If the data were not normally distributed, we used the Wilcoxon test, and results are presented as median (range). For every analysis, a P value of less than 0.05 was deemed statistically significant.

3. RESULTS AND DISCUSSION

The current investigation assessed the expression levels of the lncRNAs *HOTAIR*, *P21*, and *RP11-446E9* in patients with a positive ALL diagnosis. With an average age of less than ten years, ALL is one of the most common malignancies in children ⁽²¹⁾. Accelerated proliferation of WBCs and their immature stem cells is another defining characteristic. Based on immunological markers and clinical data, ALL is divided into multiple groups (mostly based on CD cell-surface markers):

T-cell, Pro B-cell, Pre B-cell, B-cell, and Mixed. The primary basis for this classification is blood lymphocyte markers (CD markers) ⁽²²⁾. Early detection and effective treatment methods can be highly beneficial, especially given the high death rate linked to this illness. lncRNAs are considered among the most reliable and efficient molecular indicators. These non-coding molecules are expressed at varying levels in many malignancies, including breast tumors, and play important roles in altering gene expression profiles and epigenetic changes ⁽²³⁾, prostate malignancy ⁽²⁴⁾, and ALL ⁽²⁵⁾. Alterations in lncRNA expression can trigger changes in downstream signaling pathways and alter target gene expression. Therefore, examining lncRNAs at the transcriptional and translational levels can be highly significant. Additionally, alterations in the induction of signal pathways such as RAS/MAP kinase, NF- κ B, AKT, and β -catenin can result from the expression of lncRNAs ⁽²⁶⁾.

3.1 PBMC Characterization and Clinical Evaluation of ALL Patients

Regular Complete blood count (CBC) assessments are essential for optimizing patient care and improving outcomes in individuals diagnosed with ALL. The CBC is often the first test performed when leukemia is suspected. Table 3 shows some hematological parameters of ALL patients and healthy individuals. Our findings show that mean blood parameters differ significantly ($P < 0.05$) between ALL patients and healthy individuals for blast cells (BLAST), lymphocytes (LYM), lymphocyte percentage (LYM%), mid-sized cells (MID), granulocyte percentage (GRA%), hematocrit (HCT), red blood cell count (RBC), platelet count (PLT), and hemoglobin (HB). While the mean levels of HB, GRA%, RBC, HCT, and PLT were higher in the control group than in the patient group, the mean levels of MID, LYM, LYM%, and BLAST were higher in ALL patients than in the healthy group (Table 3). Furthermore, no differences were found between the patient and control groups in the mean parameters of MCV, MCH, GRA and MID%. WBC levels in ALL

patients were somewhat greater than in healthy people, but this difference was not statistically significant. Many studies estimate a complete blood count (CBC) and most hematology parameters; these have clinical value for diagnosis or assessing certain blood conditions and for tracking treatment response⁽²⁷⁾. Depending on the disease stage and the extent of bone marrow invasion, an increase in lymphoblasts usually results in leukocytosis (high white blood cell count) or leukopenia (low white blood cell count). Moreover, anemia (low hemoglobin levels) and thrombocytopenia (low platelet counts) may arise from the squeeze-out of healthy cells⁽²⁸⁾.

Table 3: Hematological parameters of ALL patients and healthy donors.

Parameters/ Mean $\pm$ SE	Patients	Controls	P-value
WBC ($10^9/L$)	18.66 $\pm$ 4.579	8.272 $\pm$ 0.282	0.0850
Hb g/dL	9.500 $\pm$ 0.458	13.500 $\pm$ 0.263	<0.0001
Platelets ($10^9/L$)	114.1 $\pm$ 28.25	286.0 $\pm$ 8.945	<0.0001
LYM ($10^9/L$)	11.26 $\pm$ 2.932	3.358 $\pm$ 0.275	0.0080
LYM (%)	54.79 $\pm$ 4.524	39.02 $\pm$ 2.282	0.0021
MID ($10^9/L$)	3.165 $\pm$ 0.995	0.581 $\pm$ 0.033	0.0410
MID (%)	10.390 $\pm$ 1.349	6.730 $\pm$ 0.191	0.0674
GRA ($10^9/L$)	3.877 $\pm$ 0.754	4.452 $\pm$ 0.206	0.3607
GRA (%)	31.76 $\pm$ 4.602	53.89 $\pm$ 2.311	<0.0001
RBC ($10^{12}/L$)	3.337 $\pm$ 0.171	4.721 $\pm$ 0.081	<0.0001
HCT (%)	25.99 $\pm$ 1.360	38.78 $\pm$ 0.822	<0.0001
MCH (pg)	28.83 $\pm$ 0.615	28.65 $\pm$ 0.376	0.7589
MCV (fl)	80.19 $\pm$ 1.677	82.15 $\pm$ 1.090	0.1964

3.2 lncRNA Expression Profile of ALL Patients and Healthy Donors

lncRNAs have emerged as critical regulators of gene expression and are increasingly recognized for their roles in cancers, including ALL. The expression patterns of these lncRNAs may provide insight into ALL pathogenesis, prognosis, and potential therapeutic targets. This study examined the expression of three lncRNAs in ALL patients and control participants to assess differences in lncRNA expression, as shown in Figure 3. ALL patients had significantly higher levels of lncRNA *RP11-446E9*, lncRNA *HOTAIR*, and lncRNA *P21* than the control group; the corrected p-value ($P < 0.05$) indicated a significant difference.

Additionally, the results demonstrated that the expression of the lncRNAs *RP11-446E9* gene was significantly higher in ALL samples than in control samples ($P=0.0218$) (Figure 3A), that the expression of lncRNA *HOTAIR* was higher in ALL patients than in healthy individuals ($p<0.0001$) (Figure 3B), and that the expression of lncRNA *P21* was higher in ALL patients than in healthy individuals ($p=0.0252$) (Figure 3C).

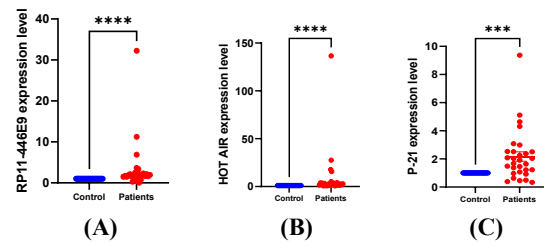


Fig. 3: Differential lncRNA expression between ALL patients and healthy controls.

A: *RP11-446E9* expression level, B: *HOTAIR* expression level, C: *P-21* expression level

Our results show that lncRNA *RP11-446E9* was upregulated, consistent with the study by Moreno et al., which found that leukemia cells' migration and proliferation rates decreased when lncRNA *RP11-446E9* was expressed at higher levels. This suggests that *RP11-446E9* plays a significant role in signaling pathways that rely on migration and proliferation. The same study showed that elevated lncRNA *RP11-446E9* expression enhances apoptosis in response to genotoxic stress⁽²⁹⁾. Patients with ALL have a different methylation status in the promoter region of *RP11-446E9* than do healthy people. Although the link is weak, there appears to be a connection between increased methylation and lower lncRNA expression, which may also serve as a biomarker for early diagnosis and prognosis in acute lymphoblastic leukemia.⁽¹⁹⁾ Moreover, lncRNA *RP11-446E9* contributes to the activation of DNA damage-induced cell death. It is not involved in the response to DNA damage; furthermore, elevated expression of this lncRNA reduces cell migration and proliferation⁽³⁰⁾.

Consistent with the present study, Eldin et al. showed that *HOTAIR* expression is much higher in ALL patients than in healthy individuals, and its

level is associated with clinical outcomes such as overall survival and event-free survival. ⁽³¹⁾. Our data showed that lncRNA HOTAIR was upregulated in ALL patients compared with the control sample, corroborating Zhang et al.; HOTAIR is commonly highly expressed in several cancer types, particularly acute lymphoblastic leukemia. Research shows that raised *HOTAIR* levels are linked to worse prognosis and shorter survival in ALL patients. ⁽³²⁾. Furthermore, HOTAIR engages chromatin remodeling complexes, such as PRC, that modify gene expression. This association represses gene expression that suppresses tumor growth, thereby allowing the development of oncogenic pathways. In ALL, *HOTAIR*'s role in modifying chromatin structure is believed to contribute to the clonal evolution of hematopoietic cells. ⁽³³⁾.

Our results disagree with studies that have reported lncRNA P21 as frequently downregulated in patients with ALL compared with healthy individuals. Downregulation of lncRNA P21 is considered a strong predictor of a poor disease course and may even promote resistance of leukemic cells to standard therapies. ⁽³⁴⁾. One function of lncRNA *p21* is to regulate the cell cycle. It can cause cell cycle arrest, especially by blocking the G1 phase; thus, it is undoubtedly crucial for the genesis of uncontrolled cell growth, a hallmark of cancer. This feature is especially relevant in ALL because disruption of cell-cycle checks is a major factor in blood cancer development. ⁽¹⁸⁾.

This reduced expression may stem from p53 being a leading candidate for suppressing apoptosis resulting from mutations and deletions in several leukemias, in addition to ALL. The possibility that lncRNA *p21* is expressed at low levels, thereby affecting the survival and growth of leukemic cells by inhibiting apoptotic pathways, cannot be excluded. ⁽³⁵⁾.

If p21 expression is not regulated, leukemic cells may grow rapidly, which can elevate WBC counts. The disruption is frequently noted in patients with ALL, where the occurrence of high WBC counts at

diagnosis is linked to a poor prognosis as well as more aggressive disease. ⁽³⁶⁾. In ALL, reduced p21 may indicate higher leukocyte counts and more severe symptoms, whereas increased p21 can promote leukemic cell death. ⁽³⁷⁾. The tumor suppressor p53 induces the lncRNA p21 and promotes apoptosis in cancer cells. It interacts with hnRNP-K. The tumor suppressor protein p53 interacts with an lncRNA termed *p21*, which affects gene expression and cellular responses to stress. This suggests that the interaction with lncRNA p21 is pro-apoptotic, indicating that lncRNA p21 acts as a tumor suppressor in ALL. ⁽³⁸⁾.

3.3 lncRNA Expression and its Correlation with Some Blood Parameters

Based on the unique biomolecular networks associated with normal and malignant states, we conducted Spearman correlation analysis. In this study, we found that blood parameters (HB, PLT, and WBC) were related to lncRNA expression. In some cases, we observed a positive correlation between lncRNA expression and each parameter, while in others, we observed an inverse correlation. The expression of lncRNA *RP11-446E9* was negatively correlated with HB and positively correlated with WBC and PLT. For lncRNA P21, Expression was positively correlated with PLT and WBC; in contrast, it was negatively correlated with HB. Additionally, for lncRNA *HOTAIR*, expression was positively correlated with HB, PLT, and WBC (Figure 4).

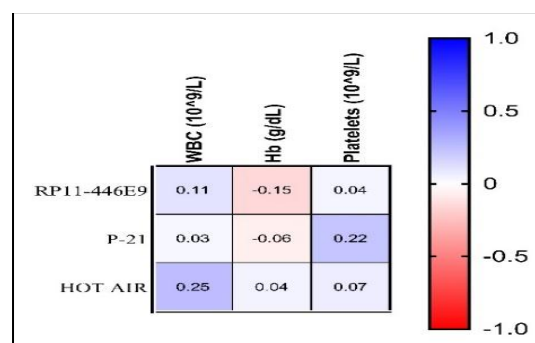


Fig. 4: Spearman correlation coefficient analysis of lncRNA expression levels with (HB, PLT, WBC)

Our results show correlations between the lncRNAs RP11-446E9, HOTAIR, and P21 and blood parameters (HB, WBC, PLT). The expression levels

of individual lncRNAs are associated with blood parameter characteristics, such as white blood cell counts, hemoglobin levels, and platelet counts. For example, overexpression of certain lncRNAs may drive leukemic cell growth and thus increase white blood cell counts. At the same time, loss of tumor-suppressive lncRNAs may also contribute to anemia and thrombocytopenia ⁽³⁹⁾.

lncRNA *p21* is a DNA sequence produced by the *p53* gene. It plays an important role in signaling cell adaptation during DNA repair and cell-cycle arrest, particularly by mediating apoptosis. In ALL, *p21*'s ability to negatively regulate cell proliferation and induce apoptosis is key to removing leukemic cells. In this state, *p21* induces apoptosis, resulting in an increase in leukemic cells due to the absence of white blood cells, which are eliminated from the circulation. ⁽³⁸⁾.

3.4 Interplay Between lncRNA and CD markers

lncRNAs strongly influence gene expression and are essential for immune cell differentiation and function, particularly with respect to CD markers. CD markers (Cluster of Differentiation markers) are surface proteins used as primary identifiers of different immune cell types and to distinguish between them. The expression of lncRNAs has been shown to correlate with the expression of specific CD markers (CD19, CD34, CD10, CD79a). Moreover, we hypothesized that correlations between lncRNA expression and CD markers could aid early cancer development, based on distinct biomolecular networks linked to normal and malignant states. In this investigation, we found a relative correlation between CD markers and lncRNA expression, with some instances showing positive correlations between long non-coding RNA and CD markers. In some instances, however, we observed an inverse relationship between CD markers and lncRNA expression. For instance, as illustrated in Figure 5, lncRNA *RP11-446E9* is positively connected with CD19, CD34, and CD79a expression, while negatively associated with CD10 expression. In contrast, lncRNA *P21* expression was positively associated with CD19, CD34, CD79a,

and CD10 expression. Furthermore, lncRNA *HOTAIR* expression was negatively associated with CD19 expression and positively connected with CD34, CD10, and CD79a, respectively.

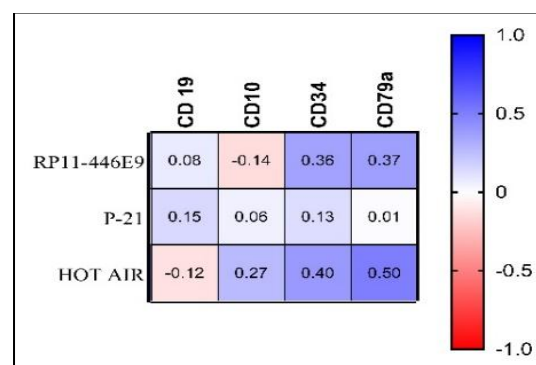


Fig. 5: Spearman correlation coefficient analysis of lncRNA expression levels with CD markers

Disrupting lncRNAs that increase CD19 and CD10 levels, which lead to B-cell precursor ALL, could be a valuable resource for the community, particularly for early diagnosis or prognosis. ⁽⁴⁰⁾ lncRNA expression profiles have been shown to distinguish different ALL subtypes, including B-cell and T-cell ALL. This discrimination is often linked to the expression of CD markers that define these subtypes. For example, lncRNAs can help differentiate B-ALL, characterized by CD19 and CD22 expression, from T-ALL, which typically expresses CD3 and CD7. ⁽⁴¹⁾.

4. CONCLUSION

In conclusion, we observed significant upregulation of lncRNA *RP11-446E9*, lncRNA *HOTAIR*, and lncRNA *P21* in ALL patients compared with controls. Additionally, we showed variation in the correlation between Long non-coding RNA expression and HB, PLT and WBC. Moreover, based on the identification of unique biomolecular networks associated with normal and malignant states, we postulated that correlations between lncRNA expression and CD markers may be useful in the early stages of cancer development. A prospective prognostic evaluation of these lncRNAs is warranted in light of the reported data. Additionally, these lncRNAs may serve as new diagnostic tools and, in the future, as prognostic biomarkers to aid treatment decisions and follow-up

in ALL patients, as well as to assess therapeutic tolerance.

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Author contribution: N.H.H. conceived the study, conducted most experiments, interpreted the results, and drafted the manuscript. Z.F.A. supervised, conceptualized, wrote, edited the manuscript, and interpreted the findings. Z.F.A. helped with experiment analysis and paper format, and the authors reviewed and approved the final product.

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