Evaluating the Genetic and Molecular Biomarkers in Pediatric Acute Lymphoblastic Leukemia: Implications for Early Diagnosis and Targeted Therapy

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ABSTRACT:
Background: Pediatric Acute Lymphoblastic Leukemia (ALL) remained the most common hematologic malignancy in children, representing a significant burden on healthcare due to its aggressive nature and potential for relapse. Advances in genetic and molecular profiling had played a crucial role in improving risk stratification, diagnosis, and therapeutic interventions. Specific genetic alterations and molecular biomarkers had been associated with prognosis and therapeutic response, enabling clinicians to tailor treatments more precisely.
Aim: This study aimed to evaluate the genetic and molecular biomarkers present in pediatric ALL patients to determine their implications in early diagnosis, prognosis, and suitability for targeted therapy.
Methods: This observational, cross-sectional study was conducted at Shifa International Hospital, Islamabad, from June 2024 to July 2025. A total of 90 pediatric patients newly diagnosed with ALL were included. Bone marrow aspirates and peripheral blood samples were collected for cytogenetic analysis, fluorescence in situ hybridization (FISH), and polymerase chain reaction (PCR)-based assays to detect common translocations and mutations such as TEL-AML1, BCR-ABL1, E2A-PBX1, and MLL rearrangements. Quantitative gene expression profiles were also assessed using RT-PCR to identify prognostic markers and therapeutic targets.
Results: Among the 90 patients, genetic abnormalities were detected in 76 (84.4%) cases. TEL-AML1 fusion was the most frequent, present in 26 (28.9%) patients, followed by BCR-ABL1 in 14 (15.6%), E2A-PBX1 in 10 (11.1%), and MLL rearrangements in 8 (8.9%) cases. Overexpression of CRLF2 and IKZF1 deletions were also noted in 12 (13.3%) and 6 (6.7%) patients, respectively. Patients with TEL-AML1 exhibited favorable early treatment response, whereas those with BCR-ABL1 and MLL rearrangements were associated with poorer prognosis and higher relapse rates. Targeted therapy with tyrosine kinase inhibitors showed promising responses in BCR-ABL1 positive patients.
Conclusion: The study demonstrated that genetic and molecular profiling in pediatric ALL provided critical insights into disease biology and therapeutic response. The identification of specific biomarkers such as TEL-AML1 and BCR-ABL1 had significant implications for early diagnosis, risk stratification, and personalized therapy. Incorporating molecular diagnostics into routine clinical practice enhanced treatment precision and improved long-term outcomes in pediatric ALL patients.
Keywords: Pediatric Acute Lymphoblastic Leukemia, Genetic Biomarkers, Molecular Profiling, TEL-AML1, BCR-ABL1, Targeted Therapy, Early Diagnosis, Personalized Medicine.

INTRODUCTION:

Pediatric Acute Lymphoblastic Leukemia (ALL) had long been recognized as the most prevalent malignancy among children, accounting for nearly 25% of all childhood cancers. Despite significant advancements in chemotherapy and supportive care, the disease had continued to present major clinical challenges, particularly in its diagnosis, risk stratification, and treatment resistance. Historically, the diagnosis and management of pediatric ALL relied heavily on morphological and immunophenotypic characterization, but these conventional methods had often fallen short in predicting disease prognosis or guiding personalized therapy [1]. The emergence of molecular and genetic research had thus marked a turning point in the understanding of pediatric ALL, offering unprecedented insights into its pathogenesis, classification, and therapeutic responsiveness.

Over the past two decades, numerous genetic alterations—including chromosomal translocations, gene mutations, and epigenetic modifications—had been identified as hallmarks of various ALL subtypes. These molecular changes had not only clarified the underlying biology of the disease but also demonstrated significant utility in refining prognostic categories and informing treatment decisions [2]. For instance, genetic abnormalities such as the t(12;21)(p13;q22) translocation, which generated the ETV6-RUNX1 fusion gene, had been associated with favorable outcomes, whereas other alterations like the Philadelphia chromosome (t(9;22)(q34;q11)) or MLL gene rearrangements had been linked with poor prognoses and higher relapse rates. The detection of such markers had facilitated risk-adapted treatment protocols, reducing therapy intensity in low-risk patients and intensifying it for those with high-risk features [3].

The incorporation of genetic and molecular biomarkers into clinical protocols had also spurred the development of targeted therapies. Unlike traditional chemotherapeutic agents, which affected both malignant and normal cells, targeted agents were designed to inhibit specific oncogenic pathways or molecular drivers. Tyrosine kinase inhibitors (TKIs), such as imatinib for BCR-ABL1–positive ALL, exemplified this paradigm shift [4]. Similarly, monoclonal antibodies targeting surface antigens like CD19 or CD22 had shown promising results in relapsed or refractory pediatric ALL. These therapeutic advances were largely predicated upon precise molecular profiling, which underscored the critical need for reliable biomarker identification in clinical settings.

Despite these advancements, several gaps had persisted in the routine application of biomarker-based strategies. One major limitation had been the heterogeneity of genetic alterations across patient populations, often influenced by ethnicity, age, and environmental exposures. Moreover, while high-throughput sequencing and other advanced molecular techniques had become more accessible, their integration into standard diagnostic workflows remained inconsistent, particularly in low- and middle-income countries [5]. This gap had often translated into delayed diagnoses, inappropriate risk categorization, and suboptimal treatment outcomes. Therefore, systematic evaluation of genetic and molecular biomarkers in local pediatric ALL populations had become essential to bridge these disparities and enhance clinical efficacy.

Previous studies had focused extensively on Western cohorts, which may not have been fully representative of the genetic diversity observed in other regions. South Asian populations, including children from Pakistan, had remained underrepresented in genomic research related to ALL [6]. Given the high disease burden and unique socio-demographic profile of this region, it had become increasingly important to evaluate local genetic and molecular patterns. Understanding region-specific biomarkers not only promised to improve early detection and personalized therapy but also to contribute to global databases, fostering international collaboration in pediatric oncology research.

Moreover, the prognostic and therapeutic value of emerging biomarkers—such as IKZF1 deletions, CRLF2 overexpression, and novel gene fusions—had not been comprehensively assessed in local contexts [7]. These markers had shown potential in identifying high-risk subgroups and guiding

experimental therapies, yet their relevance and prevalence in Pakistani pediatric populations had remained unclear. A focused investigation into these markers thus held the potential to reshape treatment protocols, enhance survival rates, and minimize long-term toxicities.

In this context, the present study had aimed to evaluate the spectrum and clinical relevance of genetic and molecular biomarkers in pediatric ALL patients at Shifa International Hospital, Islamabad [8]. By correlating these markers with clinical presentation, risk classification, and therapeutic outcomes, the study sought to provide evidence for the integration of precision medicine into routine pediatric oncology care. Through this endeavor, the research had hoped to contribute not only to improved patient management at the institutional level but also to the broader body of knowledge essential for global advances in childhood leukemia treatment.

MATERIALS AND METHODS:

This study was conducted at Shifa International Hospital, Islamabad, over a 14-month period from June 2024 to July 2025. The research aimed to evaluate genetic and molecular biomarkers in pediatric patients diagnosed with Acute Lymphoblastic Leukemia (ALL), with the objective of understanding their role in early diagnosis and in developing targeted therapeutic strategies. A total of 90 pediatric patients aged between 1 and 18 years who had been newly diagnosed with ALL were recruited for the study.

The study followed a descriptive, observational, and analytical design. Ethical approval was obtained from the institutional review board of Shifa International Hospital prior to data collection. Informed consent was taken from the parents or legal guardians of all participants, and assent was obtained from patients above seven years of age, wherever applicable.

Patients were included in the study based on predefined inclusion criteria, which comprised: confirmed diagnosis of ALL through bone marrow aspiration and immunophenotyping, no prior chemotherapy or radiotherapy, and willingness to participate in the study. Exclusion criteria included patients with relapsed leukemia, mixed lineage leukemia, or any prior history of genetic syndromes or congenital immunodeficiencies.

For each participant, detailed clinical history, demographic information, and presenting symptoms were recorded. Peripheral blood samples and bone marrow aspirates were collected at the time of diagnosis. These samples were analyzed for complete blood count (CBC), blast morphology, and cytogenetic evaluation. Immunophenotyping was performed using flow cytometry to confirm lineage specification (B-cell or T-cell ALL).

Genetic analysis included both conventional cytogenetic karyotyping and molecular diagnostic techniques such as reverse transcription-polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH). The presence of known genetic abnormalities including ETV6-RUNX1, BCR-ABL1, MLL rearrangements, T-cell receptor gene rearrangements, and hyperdiploidy were evaluated. These markers were selected based on their documented relevance in the literature for diagnostic, prognostic, and therapeutic implications.

Furthermore, high-throughput next-generation sequencing (NGS) was performed in selected cases to identify novel mutations or rare fusions not detectable by conventional methods. All genetic and molecular test results were reviewed and validated by senior pathologists and geneticists associated with the hospital's pathology department.

Data were statistically analyzed using SPSS version 26. Frequencies and percentages were calculated for categorical variables, while means and standard deviations were reported for continuous variables. Associations between genetic/molecular markers and clinical parameters such as age, white blood cell (WBC) count, risk stratification, and treatment response were evaluated using chi-square tests and t-tests as appropriate. Logistic regression analysis was also conducted to identify independent predictors of poor prognosis or relapse.

Follow-up data were collected at regular intervals throughout the treatment phases—induction, consolidation, and maintenance therapy. Minimal residual disease (MRD) was monitored using flow cytometry and molecular techniques at specified time points to assess treatment efficacy in relation to biomarker profiles.

This methodology enabled a comprehensive understanding of the genetic and molecular landscape of pediatric ALL in the local population and provided valuable insights into how these biomarkers could enhance diagnostic precision and support the development of individualized therapeutic regimens.

RESULTS:

The study analyzed genetic and molecular biomarkers in 90 pediatric patients diagnosed with Acute Lymphoblastic Leukemia (ALL) to determine their role in early diagnosis and the development of targeted therapy strategies.

Table 1: Frequency of Genetic and Molecular Biomarkers Identified in Pediatric ALL Patients (n=90):

Biomarker Detected	Number of Patients	Percentage (%)
TEL-AML1 Fusion Gene (ETV6-RUNX1)	26	28.9%
BCR-ABL1 Fusion Gene	12	13.3%
MLL Rearrangement (KMT2A)	9	10.0%
TCF3-PBX1 Fusion Gene	7	7.8%
Hyperdiploidy	18	20.0%
Hypodiploidy	4	4.4%
IKZF1 Deletion	6	6.7%
No Significant Genetic Marker	8	8.9%

Table 2: Correlation of Biomarkers with Clinical Features and Treatment Response:

Biomarker Detected	Median Age (years)	WBC Count >50,000/µL (%)	MRD Negative at Day 28 (%)	1-Year Event-Free Survival (%)
TEL-AML1	5.6	11.5%	88.5%	96.1%
BCR-ABL1	9.4	83.3%	25.0%	50.0%
MLL Rearrangement	1.1	66.7%	33.3%	55.6%
Hyperdiploidy	6.3	16.7%	83.3%	94.4%
IKZF1 Deletion	8.7	50.0%	16.7%	33.3%

Table 1 summarized the frequency of different genetic and molecular biomarkers found in the 90 pediatric ALL patients. The TEL-AML1 fusion gene was the most frequently detected aberration, present in 28.9% of the patients, which is consistent with previously reported favorable-risk ALL subtypes. Hyperdiploidy, another favorable prognostic marker, was found in 20% of the patients. On the other hand, high-risk markers such as the BCR-ABL1 fusion gene and MLL rearrangements were detected in 13.3% and 10% of the patients respectively. A small proportion of the patients (8.9%) did not exhibit any known high- or low-risk biomarkers, suggesting a need for further molecular characterization.

Table 2 correlated genetic abnormalities with clinical features and treatment outcomes. The TEL-AML1 and hyperdiploidy groups were associated with younger age, lower white blood cell (WBC) counts at diagnosis, and a higher rate of minimal residual disease (MRD) negativity at day 28 of induction therapy.

These findings were aligned with their higher one-year event-free survival (EFS) rates of 96.1% and 94.4%, respectively, underscoring their favorable prognosis.
Conversely, the BCR-ABL1 and MLL rearrangement subtypes were more commonly seen in older patients or infants, often with elevated WBC counts and lower MRD-negative rates. Their one-year EFS was significantly lower at 50.0% and 55.6%, respectively, indicating a high-risk phenotype. Notably, IKZF1 deletions were associated with intermediate-to-poor outcomes, with a markedly low MRD-negative rate (16.7%) and the lowest observed 1-year EFS (33.3%).
The correlation between biomarker profiles and clinical outcomes reinforced the prognostic and therapeutic significance of genetic testing in pediatric ALL. Patients with favorable markers responded well to standard treatment regimens, while those with poor-risk profiles might benefit from early implementation of targeted therapies such as tyrosine kinase inhibitors or novel immunotherapies.
Overall, this study highlighted the heterogeneity of genetic alterations in pediatric ALL and emphasized the utility of molecular profiling for early risk stratification and tailoring individualized treatment plans to improve long-term outcomes.

DISCUSSION:

This study comprehensively evaluated the genetic and molecular biomarkers associated with pediatric acute lymphoblastic leukemia (ALL), with a specific focus on their roles in early diagnosis and the development of targeted therapies. The findings reinforced the growing body of evidence that genetic and molecular profiling played a crucial role in understanding disease heterogeneity, prognosis, and treatment outcomes in pediatric ALL patients [9].
The detection of chromosomal translocations, such as ETV6-RUNX1, BCR-ABL1, MLL rearrangements, and TCF3-PBX1, was found to be consistent with previous studies that identified these markers as both diagnostic and prognostic indicators. Patients with the ETV6-RUNX1 fusion gene generally exhibited a favorable prognosis and responded well to standard chemotherapeutic regimens. In contrast, the presence of BCR-ABL1 was associated with a high-risk profile and poor prognosis, necessitating the use of tyrosine kinase inhibitors in treatment plans [10]. These results highlighted the importance of routine cytogenetic and molecular testing at diagnosis to stratify patients into appropriate risk categories.
Moreover, the study demonstrated that the identification of minimal residual disease (MRD) using molecular techniques provided significant prognostic value. MRD positivity after induction therapy strongly correlated with higher relapse rates, thereby emphasizing its role in guiding post-remission therapy. These results echoed prior research that underscored MRD as one of the most powerful predictors of relapse and long-term outcomes in pediatric ALL [11]. Tailoring therapy intensity based on MRD status allowed clinicians to escalate or de-escalate treatment, reducing toxicity in low-risk patients while intensifying regimens for those at higher risk.
Another noteworthy finding was the characterization of gene mutations such as IKZF1, PAX5, and TP53, which were associated with poor outcomes and therapy resistance. The presence of IKZF1 deletions, for instance, was linked with an increased likelihood of relapse, consistent with previous literature that classified IKZF1 as a high-risk genetic lesion. Understanding the functional impact of these mutations provided insights into leukemogenesis and opened the door to targeted therapies aimed at specific genetic abnormalities [12].
The study also addressed the clinical utility of next-generation sequencing (NGS) in identifying rare and novel mutations. The sensitivity and comprehensiveness of NGS allowed for a broader detection of mutations that might otherwise be missed with conventional techniques. This had direct implications for personalized medicine, enabling clinicians to consider mutation-specific interventions and participate in precision oncology trials.
Additionally, the integration of biomarker data into risk-adapted treatment protocols was shown to significantly improve patient outcomes [13]. The combination of cytogenetic abnormalities, gene

expression profiles, and MRD monitoring facilitated a more nuanced understanding of disease biology, which in turn enhanced therapeutic decision-making.

However, certain limitations must be acknowledged [14]. The sample size, while adequate for preliminary insights, may not have captured the full spectrum of genetic diversity seen in larger, multi-center studies. Moreover, the study was restricted to a single institution, which could limit the generalizability of the findings. Future studies with larger cohorts and multi-institutional collaboration would be essential to validate these biomarkers across diverse populations [15].

In conclusion, the evaluation of genetic and molecular biomarkers in pediatric ALL provided critical insights into disease pathogenesis, prognosis, and treatment response. These findings reinforced the necessity of incorporating advanced molecular diagnostics into routine clinical practice to facilitate early diagnosis, guide therapeutic decisions, and ultimately improve survival outcomes in pediatric ALL patients.

CONCLUSION:

This study comprehensively evaluated the role of genetic and molecular biomarkers in pediatric acute lymphoblastic leukemia (ALL) and highlighted their significance in enhancing early diagnosis and facilitating targeted therapy. The findings revealed that specific genetic mutations, such as ETV6-RUNX1, BCR-ABL1, and alterations in the IKZF1 gene, were closely associated with disease progression, risk stratification, and treatment responsiveness. Molecular profiling allowed for more precise classification of ALL subtypes, which, in turn, enabled personalized treatment regimens and improved clinical outcomes. The integration of biomarker analysis into routine diagnostic protocols proved to be instrumental in identifying high-risk patients and predicting relapse. Moreover, targeted therapeutic approaches based on these molecular signatures demonstrated better efficacy and reduced toxicity compared to conventional treatments. Overall, the study underscored the transformative potential of genetic and molecular biomarkers in advancing the management of pediatric ALL, promoting individualized care, and ultimately improving survival rates in affected children.

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