RESEARCH ARTICLE

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Comprehensive Analysis of *NOTCH1* Mutations in Indian Pediatric ALL Patients: Clinical and Molecular Insights

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Abstract

Background: Acute Lymphoblastic Leukemia (ALL) is a prevalent childhood malignancy characterized by abnormal lymphoid cell proliferation. Despite treatment advancements, relapse remains problematic, necessitating improved prognostic markers. *NOTCH1* mutations, particularly in T-cell ALL (T-ALL), have been implicated in ALL pathogenesis. **Methods:** We examined the correlation between *NOTCH1* mutations and clinical characteristics in 185 Indian pediatric ALL patients. *NOTCH1* mutations were detected using PCR and sequencing, with subsequent analysis of clinical parameters and outcomes. **Results:** Twenty-four cases exhibited *NOTCH1* mutations, primarily in the PEST domain, with a higher frequency in B-cell ALL (B-ALL) than in T-ALL. Specific clinical features, including elevated WBC counts and LDH levels, were associated with *NOTCH1* mutations. Survival analysis suggested potential prognostic implications of *NOTCH1* mutations in B-ALL but not in T-ALL. Integrating *NOTCH1* mutation status with minimal residual disease levels post-induction therapy may aid in identifying high-risk patients. **Conclusions:** Our findings underscore the heterogeneous nature of ALL and advocate for personalized therapy guided by molecular markers. Further research is essential to elucidate the role of *NOTCH1* mutations in ALL prognosis and treatment response.

Keywords: Prognostic Factors- Clinical Diagnosis- Clinical Characteristics- Minimal Residual Disease

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Introduction

Acute Lymphoblastic Leukemia (ALL) is primarily a juvenile disease caused by recurring genetic defects that impair precursor B-cell and T-cell development and result in aberrant cell proliferation and survival. Malignant, immature lymphoid cells proliferate excessively in the bone marrow and, in most cases, the peripheral circulation, which is a defining feature of ALL [1]. Acute lymphoblastic leukemia (ALL), the most prevalent childhood malignancy, has shown remarkable advancements in treatment over the past 60 years. Less than 10% of kids with ALL were protracted survivors in the 1960s. With modern therapy, 5-year event-free survival (EFS) and overall survival (OS) rates currently approach or exceed 85% and 90%, respectively. Relapse, on the other hand, happens in about 20% of children and is linked to a high likelihood of treatment failure and death, especially if it happens within the first 18 months of therapy. It continues to be the primary reason for cancer-related deaths in children and young people [2]. Numerous molecular markers have been found to stratify risk and predict prognosis due to the prevalence of cytogenetic changes and molecular abnormalities, which are important in the pathogenesis of ALL. *NOTCH* is a significant oncogenic driver in T-cell acute lymphoblastic leukemia (T-ALL) as it binds to an enhancer that boosts MYC expression. T-ALL is known to be driven by the oncogenic protein *NOTCH1*, and about 60% of cases have somatic mutations that activate *NOTCH1*. Children with T-ALL who have *NOTCH1* mutations have a better chance of long-term survival [3]. In this study, we want to look into potential relationships between genetic changes involving the *NOTCH1* gene along with clinical characteristics of pediatric ALL patients.

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Materials and Methods

Patients And Samples

We evaluated a total of 185 Indian pediatric patients with ALL enrolled consecutively, who had been diagnosed and treated in the Pediatric Oncology Clinic of the Regional Cancer Centre (RCC), Trivandrum, Kerala, India between May 2019 and December 2020. The diagnosis was based on the World Health Organization's classification and the patients were treated using the institutional protocol (Modified BFM ALL protocol). Patients were stratified into high-risk (HR), intermediate-risk (IR), and standard-risk (SR) groups based on protocol-defined criteria. However, correlation analysis between these risk groups and *NOTCH1* mutation status was not conducted due to incomplete stratification data. Written informed consent was obtained from the parents of the patients. The diagnosis of ALL was established through bone marrow aspiration. The investigation excluded children who were lost to follow-up and did not provide consent. We collected patient data on host variables and clinical characteristics. At the time of diagnosis, morphological, and immunophenotypic studies were carried out. Clinical parameters and laboratory findings included gender, age, bone marrow blast examination, peripheral blood blast examination, total WBC count, and lactate dehydrogenase (LDH).

The study was approved by the Institutional Review Board and Human Ethics Committee of the Regional Cancer Centre. 1-2ml BM samples were collected at the time of diagnosis. Genomic DNA was extracted using the DNAeasy Blood and Tissue Kit (ZymoResearch, USA). Biotek Take3 Micro-Volume Plates and Gen 5 version 2.09 microplate reader and imager software were used to quantify DNA, and DNA integrity was assessed by agarose gel electrophoresis.

NOTCH1 Gene Mutation Analysis

Genomic DNA was amplified using specific primers for codons HD-N, PEST region of *NOTCH1* as discussed previously [13]. PCR reactions were set up using 50-100ng template DNA, Emerald gDNA Master Mix, and two specific forward and reverse primers, triple distilled water. Then these components should be incubated under specific conditions. The primer sequences used in the present study, product length, and standardized thermal cycling conditions of the genes studied are given in the table (Table 1). The PCR reactions were performed in a 25µl reaction containing 50 ng of genomic DNA, 12.5 µl of Emerald Master Mix (Takara),1µl Forward Primer, 1 µl Reverse primer, and 5.5 µl Triple Distilled Water. The PCR condition consisted of an initial denaturation step followed by 35 cycles of denaturation, annealing, and elongation steps, and a final elongation step. To check whether the PCR generated the anticipated region of the DNA, the PCR products were checked in 1.5% agarose gel. The size of the PCR product was determined by comparing DNA markers with known base pairs. The presence of mutations was confirmed by direct Sanger's sequencing (Barcode Biosciences, Bangalore).

Statistical Analysis

SPSS, or the Statistical Package for the Social Sciences, was used to conduct all statistical analyses. Utilizing the median, numerical data were compiled (range). We utilized the Fisher's Exact Test and Mann-Whitney U test for continuous variables. With the help of frequencies and percentages, qualitative data were summarised. The Pearson chi-square test was used to calculate the significance of an association between the *NOTCH1* mutation and other discrete variables among sub groups of patients. Using Kaplan-Meier survival analysis and the log-rank test, the impact of the mutation on overall survival (OS) and relapse free survival (RFS) was calculated. For OS and RFS, the period from diagnosis to the last follow-up, or death from any cause, up to a maximum of 36 months, was calculated. A P-value of <0.05 was defined as statistically significant. It is important to note that while trends were observed in survival outcomes, most p-values did not reach statistical significance, likely due to the sample size, and should be interpreted cautiously.

Results

Association of NOTCH 1 mutation with gender and clinical parameters of pediatric ALL

In pediatric ALL, there is no significant gender predominance observed between patients with and without mutations in the *NOTCH1* gene (Table 2). However, the presence of mutations in the *NOTCH1* Ex-26 gene does lead to statistically significant differences in certain clinical

Table 1. Primer Sequences of Specific Genes

Gene	Primer Sequences	Annealing Temperature (°C)	Product Size
NOTCH1 EXON-26	Forward Primer- AGGAAGGCGGCCTGAGCGTGT	67	508 bp
	Reverse Primer- AGAGTTGCGGGGATTGACCGT		
NOTCH1 EXON-34	Forward Primer- GCTGCACAGTAGCCTTGCTG	64	631 bp
	Reverse Primer- GCGCGCCGTTTACTTGAAG		
PTPN11 EXON-3	Forward Primer: CCGACGTGGAAGATGAGATCTG	60	357 bp
	Reverse Primer: CATACACAGACCGTCATGCATTTC		
PTEN EXON-7	Forward Primer: GACAGTTAAAGGCATTTCCTG	60	265 bp
	Reverse Primer: GTCCTTATTTTGGATATTTCTCCCAATG		

Table 2. Association of *NOTCH1* Mutation with Gender of Pediatric ALL

	B-ALL (n=142)				T-ALL (n=43)			
	NOTCH1 Exon-26		*NOTCH1* Exon-34		*NOTCH1* Exon-26		*NOTCH1* Exon-34	
	Wildtype	Mutation	Wildtype	Mutation	Wildtype	Mutation	Wildtype	Mutation
Gender	Wildtype	Mutation	Wildtype	Mutation	Wildtype	Mutation	Wildtype	Mutation
Male	81	6	80	7	31	2	28	5
Female	53	2	51	4	8	2	9	1
Total	134	8	131	11	39	4	37	6
P-Value	0.485		1		0.226		1	

parameters, particularly higher white blood cell counts (WBC). Conversely, no such variations are noted in other factors like age, hemoglobin levels, platelet counts, LDH levels, or blast percentages in bone marrow and peripheral blood. Regarding *NOTCH1* Ex-34, notable differences are seen in variables such as WBC counts and LDH levels in B-ALL cases (Table 3). In T-ALL, significant discrepancies are observed in specific aspects like bone marrow blast percentage (BM BLAST), indicating the potential impact of *NOTCH1* Ex-26 mutations on disease manifestation (Table 4). Although statistically significant differences related to *NOTCH1* Ex-34 gene mutations are not widespread across most parameters in T-ALL, there is a noticeable trend in certain factors such as age and platelet counts.

Frequency of NOTCH1 mutations in pediatric ALL

The study investigated mutation hotspots within the *NOTCH1* gene, specifically targeting exon 26 and exon 34, using direct sequencing of PCR-amplified samples. It found mutations in *NOTCH1* exon 26 in 7 cases and in *NOTCH1* exon 34 in 12 cases. Additionally, mutations were detected in both *NOTCH1* exon 24 and exon 34, each occurring in 5 cases. In the B-acute lymphoblastic leukemia (B-ALL) subtype, 3 cases showed mutations in *NOTCH1* exon 26, while 6 cases exhibited mutations in *NOTCH1* exon 34, and 5 cases had mutations in both *NOTCH1* exon 26 and exon 34. Among the T-acute lymphoblastic leukemia (T-ALL) subtype, 4 cases presented mutations in *NOTCH1* exon 26, and 6 cases displayed mutations in *NOTCH1* exon 34.

NOTCH1 Mutation Profile Characteristics

Substitution mutations were prevalent in *NOTCH1* exon 26 and exon 34. Within *NOTCH1* exon 26, missense substitution mutations were detected in all 7 cases, specifically at codon 4927 with a G>C change resulting in an alteration from alanine to proline at position 1643. Similarly, in *NOTCH1* exon 34, missense substitution mutations were identified in all 12 cases, occurring at codon 7401 with a G>C alteration leading to a change from serine to tryptophan at position 2467. In the B-ALL subtype, 5 cases exhibited mutations in both exons, showcasing the same type of mutations as described above.

Survival Outcomes in Pediatric ALL According to NOTCH1 Mutation Status

In pediatric B-ALL cases, wildtype *NOTCH1* Exon-

Table 3. Association of *NOTCH1* Mutation with Clinical Parameters in B-ALL

Clinical Parameters	*NOTCH 1* Exon-26					*NOTCH1* Exon-34				
	Wildtype		Mutation		P-Value	Wildtype		Mutation		P-Value
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
AGE (Years)	5.15	3.705	6.63	5.069	0.286	5.18	3.736	5.82	4.513	0.595
WBC (X10^9/L)	30645.52	58969.655	94812.5	164998.783	0.005	34048.85	72232.669	36781.82	24071.054	0.012
Hb (gm%)	8.14	1.757	8.78	2.148	0.405	8.21	1.8	7.73	1.499	0.41
Platelet (X10^9/L)	79149.25	99941.675	79875	140172.789	0.626	82305.34	105221.297	42090.91	32066.975	0.352
LDH (U/L)	1113.26	1665.599	2086.13	2468.012	0.148	1124.58	1695.85	1695.27	2061.215	0.04
BM Blast (%)	84.68	10.939	85.57	7.934	0.705	84.77	11.111	84.2	5.287	0.144
PB Blast (%)	61.95	19.12	74.71	17.566	0.141	62.36	19.203	66.64	19.775	0.713

Table 4. Association of *NOTCH1* Mutation with Clinical Parameters in T-ALL

Clinical Parameters	*NOTCH1* Exon-26				P-Value	*NOTCH1* Exon-34				P-Value
	Wildtype		Mutation			Wildtype		Mutation		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Age (Years)	8.97	3.513	7.25	4.573	0.344	9.19	3.511	6.5	3.507	0.117
WBC (X10^9/L)	150407.69	166046.174	181500	141246.852	0.336	142278.38	151257.927	221266.67	225927.348	0.528
Hb (gm%)	9.19	2.793	7.4	1.671	0.259	9.04	2.784	8.88	2.724	0.986
Platelet (X10^9/L)	68435.9	60320.109	82250	48320.975	0.336	74729.73	61689.927	38833.33	21470.134	0.156
LDH (U/L)	2613.41	2665.244	1294.75	1220.313	0.292	2529.36	2734.148	2163.6	1029.456	0.449
BM Blast (%)	83	10.734	90.75	1.5	0.045	84.14	10.411	81.17	11.444	0.427
PB Blast (%)	70.81	19.914	78.5	13.675	0.435	72.07	19.536	69.83	19.783	0.455

26 and Exon-34 show high survival probabilities of 87% and exhibit a potential favorable trend. However, cases with mutations in these exons demonstrate even higher survival rates, hinting at a favorable prognosis [(Figure S1 (A) and S1 (B)]. In T-ALL, wildtype *NOTCH1* Exon-26 indicates a survival probability of 76.4%, slightly higher than the 75.1% seen in Exon-34. Notably, while Exon-34 mutations don't significantly impact survival, the presence of Exon-26 mutations suggests a potential influence on T-ALL survival outcomes [(Figure S2 (A) and S2(B)].

Although trends toward improved overall and relapse-free survival were observed in B-ALL cases with *NOTCH1* mutations, these differences did not reach statistical significance. For instance, a p-value of 0.066 was observed for Exon 34 in B-ALL, indicating marginal significance. In T-ALL, the observed survival differences also lacked statistical significance (e.g., p=0.124 for Exon 26), suggesting that these trends should be interpreted cautiously.

In B-ALL, mutations in *NOTCH1* Exon-26 and Exon-34 correlate with higher relapse-free survival rates compared to wild-type cases, suggesting a potential influence of *NOTCH1* gene status on B-ALL outcomes. A notable p-value of 0.066 for *NOTCH1* Exon-34 indicates marginal significance [(Figure S3 (A) and S3(B)]. In T-ALL, similar patterns persist, with mutations in *NOTCH1* Exon-26 and Exon-34 showing higher relapse-free survival rates than wild-type cases, highlighting the potential role of *NOTCH1* mutations in T-ALL outcomes. P-values of 0.124 for *NOTCH1* Exon-26 and 0.235 for *NOTCH1* Exon-34 suggest trends that needs further investigation [(Figure S4 (A) and S4(B)].

Discussion

Children with acute lymphoblastic leukemia (ALL) have recorded survival rates of over 80%, but less than 50% of adults with ALL respond to modern therapy. In an effort to improve patient quality of life as well as ALL cure rates, acute morbidity, and long-term consequences are being addressed as part of the current risk-directed therapy. The mechanisms of leukemic cell transformation, the evolution of drug resistance, as well as the effects of germline genetics on a patient's response to chemotherapy, are all emerging progressively comprehended. As a result, researchers are finally coming to an era of personalized therapy for ALL, in which therapies will be based on specific molecular targets and individualized treatment, as opposed to standard protocols for large patient cohorts. *NOTCH1* is a frequently mutated gene, and its abnormal expression may play a role in the pathogenesis of T-ALL in addition to the standard prognostic variables including WBC count, age, sex, immunophenotype, LDH, BM Blast, and PB Blast. Although *NOTCH1* is highly conserved across species, it has been discovered that the *NOTCH1* signaling pathway plays a role in crucial hematopoiesis processes such as T and B lineage selection, commitment to lymphocyte lineage, and subsequent lymphocyte differentiation.

In our analysis of 185 pediatric ALL patients, B-ALL cases accounted for 77% of the cases, outnumbering T-ALL cases, which comprised 23%, a distribution somewhat similar to findings described by Onciu M [4]. Pediatric ALL cases predominantly occurred in children aged 0 to 4 years, with a slight male predominance (120

males and 65 females, M: F ratio = 1.84:1), consistent with earlier observations by Woo et al. [5]. Our research identified *NOTCH1* mutations in 24 cases, a notably lower count compared to previous studies reporting a higher prevalence of *NOTCH1* mutations [6-8]. This discrepancy in mutation rates may be attributed to variances in racial demographics across different geographical regions. Notably, our analysis focused specifically on two critical segments of the *NOTCH1* gene, HD-N (Exon-26) and PEST (Exon-34), recognized as mutation hotspots. Interestingly, we observed a significantly higher occurrence of mutations in the PEST (Exon-34) region, comprising roughly 50% of all mutations. In contrast to investigations of T-cell acute lymphoblastic leukemia (T-ALL) in other populations, where both HD-N and PEST domain mutations are commonly identified, our analysis revealed only five cases with mutations in both domains, contradicting previous reports [9, 10]. Our study underscores the greater prevalence of mutations in the PEST domain compared to the HD domain, consistent with findings reported by Bhanushali et al. [11].

Out of the 24 mutations we identified, 58% were observed in pediatric B-cell acute lymphoblastic leukemia (B-ALL) cases, while the remaining 42% were in B-ALL cases, contradicting earlier reports indicating a higher frequency of *NOTCH1* mutations in T-ALL. When comparing pediatric ALL subtypes, our study revealed a significant increase in the occurrence of *NOTCH1* HD-N (p value=0.0051) and PEST region (p value=0.0006) mutations. Gender did not exhibit any correlation with *NOTCH1* mutations in our study population, as both males and females showed varying proportions of *NOTCH1* mutant cases. Similarly, there was no statistically significant correlation between *NOTCH1* mutations and patient age. However, within our study cohort, pediatric B-ALL patients with *NOTCH1* HD mutations displayed higher initial white blood cell (WBC) counts (P=0.005), while those with *NOTCH1* PEST mutations had elevated LDH levels (P=0.012) and notably increased bone marrow blast levels (p value=0.040). In T-ALL patients with NOTCH HD mutations, significantly higher BM blast levels were observed (P=0.0545). Despite substantial variations in median values between *NOTCH1* mutant and wildtype patients, no other clinical variables, such as hemoglobin, platelet count, and peripheral blood blast percentage, showed a statistically significant association with *NOTCH1* mutation status in both subtypes. The majority of cases involved single mutations, with only five patients showing both mutations simultaneously. These mutations were predominantly situated in the PEST domain, followed by the HD domain. Raouf et al. [12] conducted a study that documented a higher occurrence of mutations in the HD domain compared to the PEST domain, a finding consistent with studies by Jenkinson et al. [13]. It has been established that mutations in these two domains of the *NOTCH1* receptor lead to an increase in *NOTCH1*-dependent signal transduction. In contrast to previous research on pediatric ALL, which often reported a significant number of insertion/deletion mutations, our analysis primarily detected missense mutations [14]. The variation in the frequency, types of mutations, and affected exons of *NOTCH1* mutations may be attributed to racial diversity.

In our study of B-cell acute lymphoblastic leukemia (B-ALL), we observed that cases positive for *NOTCH1* mutations in the PEST domain showed a trend towards increased significance in terms of overall survival (OS) compared to cases with mutations in the HD domain when compared with their wildtype counterparts. This trend was similarly noted in cases of relapse-free survival (RFS), where *NOTCH1* mutations in the PEST domain exhibited marginal significance compared to cases with mutations in the HD domain. This finding supports prior research emphasizing the significance of *NOTCH1* mutations, particularly in the PEST domain, in predicting B-ALL prognosis [15] . The marginal significance observed in RFS for PEST domain mutations further underscores their potential relevance in disease progression and relapse. Although *NOTCH1* mutations in B-ALL showed trends toward improved survival outcomes, these did not reach statistical significance (e.g., p=0.066 for Exon 34), potentially due to sample size limitations. Further validation in larger, multicenter cohorts is necessary to confirm these findings. These findings highlight the necessity for further investigation into the mechanisms by which PEST domain mutations may influence the clinical outcomes of B-ALL patients. However, in T-cell acute lymphoblastic leukemia (T-ALL), we did not observe significant associations of *NOTCH1* mutations with OS and RFS. This contrasts with existing literature documenting the association of *NOTCH1* mutations with disease initiation and progression. The lack of correlations with survival outcomes in our cohort suggests that additional factors, such as genetic co-mutations or treatment modalities, may influence the prognosis of T-ALL.

The relapse-free survival outcomes in patients with *NOTCH1* gene mutations were comparable to their overall survival rates [16]. The relapse-free survival outcomes in patients with *NOTCH1* gene mutations were comparable to their overall survival rates. Interestingly, none of the T-ALL patients who relapsed exhibited *NOTCH1* mutations, suggesting that relapse in this subtype may be driven by alternative genetic alterations or microenvironmental influences, rather than *NOTCH1*-dependent mechanisms. This finding could have important implications for risk stratification and therapeutic targeting in T-ALL. Furthermore, our dataset did not include quantitative data on minimal residual disease (MRD) levels following induction therapy, nor did it capture detailed information on central nervous system (CNS) involvement. These factors are now considered critical in modern pediatric ALL risk assessment and are routinely used to guide treatment intensity. The lack of these data represents a limitation of the study and prevented us from conducting deeper correlations between *NOTCH1* mutation status, MRD burden, and CNS relapse risk in T-ALL. Future studies incorporating these variables will be essential to validate and extend our observations.

The absence of *NOTCH1* mutations in the eight relapse cases among T-cell acute lymphoblastic leukemia (T-ALL) patients is noteworthy. It suggests that while *NOTCH1*

mutations may contribute to the initial development of T-ALL, they may not be the primary drivers of relapse in this subgroup. This finding underscores the heterogeneity of T-ALL and emphasizes the necessity for further research to elucidate the factors influencing relapse in this specific context. Additionally, the similarity observed in relapse-free survival and overall survival outcomes for patients with *NOTCH1* gene mutations in T-ALL underscores the importance of vigilance and management of relapse risk in all T-ALL patients, regardless of their *NOTCH1* mutation status.

The assessment of pediatric ALL-BFM 2000 revealed favorable clinical outcomes for T-cell acute lymphoblastic leukemia (T-ALL) patients with *NOTCH1* mutations, as documented by Briet et al. [17]. The disparity in prognostic implications could be partially attributed to differences in treatment strategies. Prior studies reported lower survival rates for T-ALL patients with *NOTCH1* mutations, such as a 65% 5-year disease-free survival [8, 10], compared to the ALL-BFM 2000 study (90% relapse-free survival) and our investigation (100% 3-year event-free survival or EFS). Consequently, there remains significant uncertainty regarding the long-term survival implications of *NOTCH1* mutations in pediatric T-ALL across diverse therapeutic approaches. Patients in our cohort were treated under the modified BFM 2000 protocol and stratified into high-risk (HR), intermediate-risk (IR), and standard-risk (SR) groups; however, due to data limitations, we were unable to statistically correlate mutation status with specific risk stratification groups.

Various treatment protocols, such as BFM 2000 [18] , MRC UKALL 2003 [13] , and Japan Association of Childhood Leukemia Study ALL-97 [19] , have indicated that *NOTCH1* mutations are linked with a favorable prognosis, although other studies have not consistently supported these results. Moreover, high-risk pediatric T-cell acute lymphoblastic leukemia (T-ALL) patients with *NOTCH1* mutations have been documented to have a less favorable prognosis and a higher occurrence of central nervous system (CNS) relapses, as highlighted in the European Organization for Research and Treatment of Cancer study [20].

Yuan et al. suggest that the status of the *NOTCH1* gene and minimal residual disease (MRD) after induction therapy have emerged as potential prognostic markers. Combining the presence of *NOTCH1* mutations with an MRD level of ≤1×10-4 identified a subgroup with an impressive 100% overall survival (OS), event-free survival (EFS), and disease-free survival (DFS). These results suggest that *NOTCH1* mutations could be valuable indicators for identifying pediatric ALL patients at risk, who may potentially benefit from a favorable prognosis [21].

In conclusion, this is the largest cohort study of pediatric ALL patients from South India who were treated in a single hospital, focusing on the clinical and prognostic implications of *NOTCH1* mutations. Long-term outcomes in patients with or without *NOTCH1* mutations were the main focus of our study. Our results suggest that *NOTCH1* mutations, particularly in the PEST domain, may be associated with favorable survival trends in B-cell ALL (B-ALL). However, these associations did not reach statistical significance, underscoring the need for cautious interpretation and validation in larger cohorts.

In contrast, *NOTCH1* mutations in T-cell ALL (T-ALL) did not show clear prognostic value in our cohort. Notably, none of the relapsed T-ALL cases harbored *NOTCH1* mutations, suggesting they may not be central to relapse biology in this subtype.

While our findings support the potential utility of integrating *NOTCH1* mutation status into risk assessment especially when combined with post-induction minimal residual disease (MRD) levels we acknowledge that MRD and CNS involvement data were unavailable in this study. Similarly, we were unable to correlate mutation status with BFM-defined risk stratification groups due to incomplete data.

Overall, this study contributes important regional data to the understanding of *NOTCH1* mutations in pediatric ALL. Future multi-center studies with comprehensive molecular, MRD, and clinical stratification data are necessary to validate and extend these findings toward personalized treatment approaches.

Author Contribution Statement

PA performed molecular experiments and drafted the manuscript. TP provided clinical data. GP, RCV, TVA, JAG, MSM, and PD contributed to manuscript review and editing. NMKKJ conducted statistical analysis. SLS facilitated sample acquisition. HS supervised the project and finalized the manuscript. All authors reviewed and approved the final version.

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Statement Of Ethics

This study was approved by the Institutional Human Ethics Committee of the Regional Cancer Centre, Trivandrum, Kerala, India (HEC No. 05/2019). Informed consent was obtained from parents or guardians of all participating children.

Data Availability

Data underlying the findings of this study are available from the corresponding author upon reasonable request.

Registration

This study was not registered in a clinical trial or meta-analysis database, as it was an observational molecular analysis study.

Conflict Of Interest

The authors declare no conflict of interest.

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