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Clinico-haematological Profile and Post-Induction Remission Status of Newly Diagnosed Paediatric Acute Lymphoblastic Leukaemia Patients with ETV6::RUNX1 Gene Rearrangement

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Abstract

Objectives: To determine the frequency of ETV6:RUNX1 gene rearrangement in paediatric acute lymphoblastic leukaemia in Pakistan and to determine the clinico-haematological profile and post induction remission status of newly diagnosed paediatric acute lymphoblastic leukaemia and compare them across gender and based on ETV6::RUNX1 gene rearrangement positivity.

Methodology: This descriptive cross-sectional study was conducted at Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, Oct 2021 to Aug 2023. The study sample was composed of 229 cases of paediatric acute B-cell lymphoblastic leukaemia. Patients who had B-ALL occurring as a second malignancy, Down's syndrome, those who had received chemotherapy or corticosteroids were excluded. Patients ETV6:RUNX1 gene rearrangement status was determined by PCR. All patients underwent induction with the UK ALL 2019 protocol. Bone marrow aspiration and trephine was conducted on Day+29 of induction to assess remission and MRD status.

Results: Our study sample had a median age of 3.0 (IQR: 4.0) years, 136 (59.4%) were male. ETV6::RUNX1 gene rearrangement was seen in 36 (15.7%) cases. There were no statistical differences with regards to clinico-haematological profile at presentation and outcomes between genders and among patients with and without ETV6:RUNX1 gene rearrangement, except for MRD which had a higher frequency of being negative in the former, (p=0.023).

Conclusion: ETV6::RUNX1 rearrangement is associated with a higher frequency of negative MRD post-induction.

Keywords: Clinico-Haematological Profile, ETV6:RUNX1 Gene Rearrangement, Paediatric Acute Lymphoblastic Leukaemia, Post-Induction Remission Status.

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Introduction

Acute lymphoblastic leukaemia (ALL) is the most common childhood neoplastic disease, representing approximately a quarter of all paediatric cancer diagnoses.[1] Within this heterogeneous disorder, the presence of specific genetic abnormalities plays a pivotal role in determining clinical behavior and treatment outcomes.[2] One such genetic alteration is the ETV6::RUNX1 gene rearrangement, resulting from the reciprocal t(12;21)(p13;q22) translocation.[3] This rearrangement leads to the formation of a fusion transcript involving the ETV6 (TEL) and RUNX1 (AML1) genes and is found in approximately one-fifth of paediatric ALL patients, specifically those with B-cell ALL (B-ALL).[3]

This translocation is known to develop during intrauterine life, and is thought to be associated with the presence of a pre-leukemic phase.[4] Its activity results in the over-expressions of Recombination-Activating Gene-1 (RAG-1) and RAG recombinase activity.[5] This causes modifications of the process of differentiation of hematopoietic progenitor cells as well as an enhancement of their ability for self-renewal, especially of B-Lymphocyte lineage, which may account for its role in leukemogenesis.[6] This

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mutation was generally thought to be associated with a favourable outcome in paediatric ALL,[7] however, this prognostic benefit is not clearly demonstrated across all studies, and the rearrangement may be associated with late relapses in about one-fifth of patients, raising doubt about whether it is an indicator of good prognosis.[3,8] Additionally, it has been noted that paediatric ALL associated with this translocation has enhanced sensitivity in vitro to chemotherapy with drugs such as L-asparaginase, doxorubicin, etoposide and prednisone when compared to ALL with other cytogenetic abnormalities, which may have a bearing on treatment outcomes.[9,10]

Evaluation of the impact of post-induction chemotherapy remission status, specifically, the rate of complete remission (CR) achievement after the induction phase of chemotherapy is a critical prognostic factor for overall survival and event-free survival in ALL.[8-10] ETV6::RUNX1 gene rearrangement may be associated with different response rates to standard induction therapy, indicating a potential role in risk stratification and treatment decision-making in the South Asian population, a subject hitherto largely unstudied. Understanding the clinical and haematological characteristics associated with the ETV6::RUNX1 translocation is of paramount importance in this demographic, as it may help identify patients who require tailored therapeutic strategies for achieving optimal treatment outcomes. Additionally, investigating the impact of this genetic alteration on post-induction chemotherapy remission status holds promise for further refining risk stratification and personalized treatment approaches for our patients.

Methodology

This study was conducted as a descriptive cross-sectional study between Oct 2021 and Aug 2023 in the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi on 229 paediatric patients who had developed B-ALL, whose parents/guardians provided informed/written consent for enrollment in the study. The study was conducted in accordance with the Declaration of Helsinki and ethical guidelines. Ethical committee of Armed Forces Institute of Pathology, Rawalpindi gave the approval for this study.

We used consecutive, non-probability sampling to select participants for our research. The WHO sample size calculator was used to calculate the sample size keeping a confidence level (1-α) of 95%, an absolute precision of (d) of 0.05, and an anticipated population proportion (P) of 0.182,[3] which was the proportion of paediatric B-ALL, which was positive for the ETV6::RUNX1 gene rearrangement, from Qiu et al.[3]

Patients aged 18 years or less, of both genders, who were newly diagnosed as suffering from B-ALL, as diagnosed per the International Consensus Classification (ICC) criteria based on morphology, immunophenotyping, cytogenetics, and molecular genetics.[11]

Patients who had T-cell ALL (T-ALL), mixed phenotypic leukemia, malignancy secondary to immunodeficiency, B-ALL occurring as a second malignancy, those who were suffering from Down's syndrome, those who had received chemotherapy in any form or duration or those who had received corticosteroids within the previous month before enrollment; were excluded from the study.

Patients were documented for demographic and history data at the time of enrollment, such as age, gender and the presence of B symptoms. A physical examination followed which recorded parameters such as the presence of splenomegaly and hepatomegaly. All patients underwent testing for complete blood counts, Bone Marrow Aspiration and Trephine (BMAT) with immunophenotyping, flow cytometry, FISH for BCR/ABL and KMT2A rearrangement while ETV6::RUNX1 gene rearrangement status was determined by Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR). Patients were stratified according to the National Cancer Institute (NCI) risk criteria.[12] All patients underwent induction with the UK ALL 2019 protocol, with treatment regimen determined by their risk status. This included intrathecal chemotherapy, which was when status of Central Nervous System (CNS) involvement was determined. BMAT was repeated on Day+29 of induction to assess remission status, with determination of Minimal Residual Disease (MRD) status at this time. Complete Remission (CR) was said to have been established if <5% lymphoblasts were identified on BMAT sample, while patients were said to have CR with incomplete count recovery (CRi) if blood counts did not normalize.[3] Additionally, MRD was said to be negative if it was ≤0.01%, as determined by flow cytometry.[3]

Data was analyzed using the Statistical Package for the Social Sciences version 27.0. Mean and standard

deviation was calculated for quantitative variables specifically age, peripheral blood total leucocyte/platelet/blast counts and haemoglobin at diagnosis. Qualitative variables like gender, presence of B symptoms, presence of splenomegaly, hepatomegaly, lymphadenopathy, BCR/ABL status, KMT2A status, ETV6::RUNX1 status, NCI risk status, CNS involvement, remission and MRD status post-induction were recorded in terms of frequency and percentage. Patients were divided into two groups based on the presence of ETV6::RUNX1 gene rearrangement. Qualitative data was compared between groups using the Chi Square test/Fischer Exact test, while quantitative variables were compared using the independent samples *t* test/Mann-Whitney U test. A *p* value of ≤0.05 was considered significant.

Results

Our research was based on 229 paediatric patients who were under work-up for B-ALL in our institute. A total of 136 (59.4%) patients were males, and these patients had a median age of 3.0 (IQR: 4.0) years, with 149 (65.0%) patients aged 5 or below at the time of diagnosis. B-symptoms were present in 160 (69.9%) patients, 74 (32.3%) had splenomegaly, while hepatomegaly and lymphadenopathy were seen in 48 (21.0%) and 65 (28.4%), respectively. With regards to haematological parameters at presentation, the median total leucocyte count (TLC) was 27.5 (IQR: 36.9) x 10^3/µL, the platelet count was 43.2 (IQR: 42.2) x 10^3/µL, the mean haemoglobin level was 8.5 (IQR: 4.7) g/dL, while the median percentage of blasts in peripheral blood was 80.0 (IQR: 57.5) % at the time of presentation. A total of 13 (5.7%) cases had CNS involvement at the time of diagnosis. Table-I shows the patient characteristics at the time of diagnosis distributed according to gender.

Table II shows the results of frequencies different cytogenetic abnormalities in our study sample. BCR/ABL translocation was present in 11 (4.8%) cases, while KMT2A gene rearrangement was seen in 17 (7.4%) patients. ETV6::RUNX1 gene rearrangement was seen in 36 (15.7%) cases.

Table III shows the patients stratified according to risk status as well as post-induction outcomes. A total of 218 (95.2%) achieved remission at the end of the induction period, while 11 (4.8%) did not. MRD was negative in 194 (84.7%) patients, however, it was positive in 35 (15.3%) cases at the end of the induction period.

Table IV shows the comparison of different clinico-pathological parameters as well as outcomes between patients who were ETV6::RUNX1 gene rearrangement positive, versus those who were not. Patients who were

Table II: Frequencies of cytogenetic abnormalities .(n=229)

Variable	Males (n=136)	Female (n=93)	*p*-value
BCR/ABL translocation positivity	4 (29.4%)	7 (7.5%)	0.126
KMT2A gene rearrangement positivity	8 (5.9%)	9 (9.7%)	0.282
ETV6::RUNX1 gene rearrangement positivity	20 (14.7%)	16 (17.2%)	0.610

Table III: Risk stratification and post-induction outcomes. (n=229)

Variable	Males (n=136)	Female (n=93)	*p*-value
National Cancer Institute risk status			
Standard risk	103 (75.7%)	69 (74.2%)	0.791
High risk	33 (24.3%)	24 (25.8%)	
Remission status			
In remission	129 (94.9%)	89 (95.7%)	1.000
Not remission	7 (5.1%)	4 (4.3%)	
Minimal residual disease status			
Positive	119 (87.5%)	75 (80.6%)	0.157
Negative	17 (12.5%)	18 (19.4%)	

Table I: Patient characteristics at diagnosis (n=229)

Variable	Males (n=136)	Female (n=93)	*p*-value
Age (years)	4.0 (IQR: 4.0)	3.0 (IQR: 5.00)	0.514
B-Symptoms	96 (70.6%)	64 (68.8%)	0.774
Splenomegaly	50 (36.8%)	24 (25.8%)	0.082
Hepatomegaly	29 (13.9%)	19 (20.4%)	0.870
Lymphadenopathy	39 (28.7%)	26 (27.9%)	0.906
Total leucocyte count (10^3/µL)	25.5 (IQR: 36.7)	28.7 (IQR: 35.7)	0.536
Platelet count (10^3/µL)	41.8 (IQR: 38.3)	45.7 (IQR: 42.6)	0.635
Haemoglobin level (g/dL)	8.8 (IQR: 4.9)	8.2 (IQR: 4.2)	0.498
Blasts in peripheral blood (%)	75.0 (IQR: 95.0)	80.0 (IQR: 90.0)	0.182
Central Nervous System Involvement	6 (4.4%)	7 (7.5%)	0.317

Table IV: Clinico-pathological features and outcomes with ETV6::RUNX1 positivity.. (n=229)			
Variable	ETV6::RUNX1 positive (n=36)	ETV6::RUNX1 negative (n=193)	*P* value
Age (years)	2.5 (IQR: 6.8)	3.0 (IQR: 4.0)	0.925
Presence of B-symptoms	21 (58.3%)	139 (72.0%)	0.100
Splenomegaly	13 (36.1%)	61 (31.6%)	0.596
Hepatomegaly	9 (25.0%)	39 (20.2%)	0.517
Lymphadenopathy	10 (27.8%)	55 (28.5%)	0.930
Total leucocyte count (10^3/µL)	30.7 (IQR: 40.1)	25.9 (IQR: 36.4)	0.572
Platelet count (10^3/µL)	41.8 (IQR: 52.1)	43.2 (IQR: 39.8)	0.984
Haemoglobin level (g/dL)	8.9 (IQR: 4.8)	8.4 (IQR: 4.7)	0.728
Blasts in peripheral blood (%)	80 (IQR: 62.8)	80 (IQR: 57.5)	0.579
Central Nervous System Involvement	2 (5.6%)	11 (5.7%)	1.000
National Cancer Institute risk status			
Standard risk	24 (66.7%)	148 (76.7%)	0.202
High risk	12 (33.3%)	45 (23.3%)	
Remission status			
In remission	36 (100%)	182 (94.3%)	0.142
Not remission	-	11 (5.7%)	
Minimal residual disease status			
Positive	1 (2.8%)	34 (17.6%)	0.023
Negative	35 (97.2%)	159 (82.4%)	

positive for this rearrangement appeared to have a similar clinico-pathological profile on presentation to those who did not, but there was a difference in terms of outcomes: all of these patients were able to achieve remission after induction but the difference between groups did not achieve statistical significance and patients with ETV6::RUNX1 gene rearrangement had a significantly higher chance of having a negative MRD post-induction than those who did not, (p=0.023).

Discussion

The results presented in this study provide valuable insights into the clinical and hematological characteristics of paediatric B-ALL patients harbouring the ETV6::RUNX1 gene rearrangement and their subsequent response to post-induction chemotherapy. Paediatric B-ALL is a commonly encountered disease in Pakistan, with ETV6::RUNX1 gene rearrangements identified in approximately 15.0% of the population in our study.

Our study population was composed of a majority of males, accounting for three-fifths of all patients, and there was no difference between both genders with regards to presence of ETV6::RUNX1 rearrangement. This is in keeping with existing studies on the subject such as Kakaje et al, who also reported a male preponderance of 60.9%, in their study of patients with ALL,[13] while Wimalachandra et al also reported the same.[14] The reason for this skewed distribution is unclear, but may be attributable to the presence of certain genes on chromosome 9 close to the ABO gene locus on chromosome 9, which may be sex-steroid responsive and protective against ALL in women.[15] In addition, Bhojwani et al also confirmed that ETV6::RUNX1 rearrangement frequency did not change across gender, (p=0.53).[16]

In the current study, most of the patients had an onset of disease at the age of 5 years and below, and there was no difference between genders and the presence of ETV6::RUNX1 rearrangement with regards to age of onset. Bernaldez-Rios et al also reported that the majority of children suffering from B-ALL in their study presenting between 2 and 3 years of age,[17] while Hossain et al reported that 46% of all diagnoses of paediatric B-ALL were made between the ages of one and four years in their study sample, which was in agreement with our results.[18] Neither studied reported any difference between gender and the frequency of B-ALL.[17,18] Aljamaan et al also confirmed that ETV6::RUNX1 rearrangement had no effect on age of onset, (p=0.088).[19] However, Bhojwani et al reported that patients below the age of one years had a lower frequency of the presence of this mutation, (p<0.001).[16] We believe this difference has occurred due to the very low number of children from this age group in the latter study, and a larger sample from this population requires study before adequate conclusions can be drawn.

Our study showed that there was no difference with regards white cell count at presentation between patients who were ETV6::RUNX1 positive and those who were not. Both Bhojwani et al and Aljamaan et al also determined that white cell count was not significantly lower in patients who ETV6::RUNX1 positive, (p=0.51 and p=0.63, respectively).[16,19]

CNS involvement was not significantly different across gender or ETV6::RUNX1 positivity in the current study. Xu et al reported in their study that CNS involvement was not significantly different across gender in their study, (p=0.798), nor did ETV6::RUNX1 positivity result in lower CNS involvement, (p=0.767), which was in accordance with our result.[20] Conversely, Bhojwani et al reported that patients with ETV6::RUNX1 rearrangement had a lower incidence of CNS involvement compared to those without it, (p<0.001).[16] We believe this difference in results needs further investigation before concrete conclusions can be drawn.

There was no difference between genders, and ETV6::RUNX1 status in terms of attainment of remission post-induction, but MRD negativity was associated with a positive ETV6::RUNX1 status, in the present study. Aljamaan et al also reported that there was no difference between patients who were ETV6::RUNX1 positive and those who were not at the end of induction in terms of attaining remission, (p=0.739).[19] Bhojwani et al noted that MRD negativity was significantly associated with MRD negativity, (p=0.010), as in our study.[16] Jovanovska et al concluded that gender did not affect the MRD status post-induction, (p=0.523), which was also in accordance with our study.[21]

Limitations: We conducted our research study in a single center and, while it is a center-of-excellence which receives patients from all over the country, especially the north, a multi-center study would be more appropriate to determine whether our results are generalizable to the rest of the population. Our study was also limited by its sample size which was comparatively smaller compared to other studies conducted on the subject, and a comparison between a larger population of ETV6::RUNX1 rearranged and non-rearranged patients may yield different results. Lastly, our study did not take into account time since onset of disease manifestations which may have had an effect on the development of visceromegaly, lymphadenopathy and degree of bone marrow involvement, which may have had an indirect affect on our results.

Conclusion

ETV6::RUNX1 gene rearrangement is a commonly seen genetic abnormality in the paediatric population in Pakistan. It appears to have a positive impact on the outcomes of induction in this population of patients and a case can be made to incorporate the routine testing of this rearrangement in such patients at the time of diagnosis to determine prognosis. Further multi-center research across a more diverse population group in imperative to establish whether our results are applicable to the population-at-large.

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