Original Research Article

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Cytogenetic and molecular aberrations in childhood B lineage acute lymphoblastic leukaemia

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ABSTRACT

Background: Acute Lymphoblastic Leukemia (ALL) is a common hematological malignancy in children and is characterized by genetic changes such as mutations and chromosomal translocations. These cytogenetic and molecular abnormalities have got diagnostic and prognostic significance. Identification of these abnormalities helps in risk categorization and appropriate therapy. Aim of the study was to assess the cytogenetic/molecular abnormalities associated with B Lineage ALL in children.

Methods: It was a hospital based retrospective observational study of 79 children diagnosed with B Lineage ALL by Bone marrow aspirate morphology and flow cytometry. Bone marrow samples or Peripheral blood were sent for cytogenetic/molecular analysis by Fluorescent in situ Hybridization technique. Descriptive data analysis was done using SPSS software.

Results: Out of 199 cases 163(82%) were B Lineage ALL. 79(48%) undergone molecular analysis. Out of 79 cases of B lineage ALL, Translocation t(9;22) BCR-ABL1 was positive in 2(2.5%) cases, Translocation t(12;21) TEL/AML1 was positive 9(11%) cases and MLL (KMT2A) Gene Rearrangements was seen in 6(7.6%) children. Out of 79 cases of B lineage ALL, 6(7.6%) were Infantile ALL (Males 1(17%); Females 5(83%)). 4(67%) cases were positive for MLL (KMT2A) Gene Rearrangement, all of them were female children. Over all 17(22%) cases (Males 4(24%); Females 13(76%)) were positive for molecular abnormalities.

Conclusions: Many children with ALL have got Cytogenetic and Molecular abnormalities. The highest percentage of cytogenetic and molecular genetic abnormalities was related to t(12;21)TEL/AML1 in B Lineage ALL children, if present confer favourable prognosis. MLL (KMT2A) Gene Rearrangement was the common molecular abnormality in Infantile B ALL, presence of it leads to high risk categorization and confer poor prognosis. The evaluation of cytogenetic and molecular genetic abnormalities in children is essential in estimating the prognosis in B Lineage ALL children, which will be a great contribution to offer appropriate therapeutic approaches.

Keywords: Fluorescence in situ hybridization, Leukaemia, Translocation

INTRODUCTION

Leukemia's are the most common cancers affecting children accounting for 32% of all cancers in children younger than 15 years. Of this Acute Lymphoblastic Leukemia (ALL) accounts for 73%. Acute Lymphoblastic Leukemia (ALL) is a clonal disease of the hematopoietic system characterized by unique genetic characteristics, mutations and chromosomal

translocations.¹ The main cause of the disease remains largely unknown, but it has been shown that various factors, including environmental factors, viral infections, and genetic changes, and association with syndromes such as Down, Bloom and Congenital immune deficiency disorder like Wiscott Aldrich syndrome have been shown to occur.^{2,3} However, chromosomal translocations and related molecular variations have been shown to play a major role in pathogenesis and therapeutic response in

patients. Most of these changes occur in genes that play essential roles in lymphoid development, cell cycle, or as tumor suppressors or oncogene. Genetic changes and clinical symptoms can be useful in the classification of ALL to subtypes as well as diagnostic and prognostic monitoring.^{1,4} factors for patients Identifying chromosomal translocations and related molecular changes not only identify leukemia cells pathogenicity, but also optimize therapeutic approach to increase patient survival. In 80% of pediatric Acute Lymphoblastic Leukemia (ALL) cases, specific genetic alterations can be found in leukemic blasts using routine karyotype analysis and molecular techniques, such as Fluorescence in Situ Hybridization (FISH), Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), and Southern blot analysis. Molecular studies identify translocations more rapidly and those not detected on routine karyotype analysis and distinguish lesions that appear cytogenetically identical but are molecularly different. Important diagnostic, therapeutic, and prognostic implications are associated with the abnormalities described. Therapeutic approaches are usually based on the prognostic characteristics of chromosomal translocations and their classification based on standard, intermediate or high-risk groups.

B-lineage ALL has a variety of chromosomal abnormalities, which in almost half of cases confer favorable outcomes like t(12;21)TEL/AML 1 (ETV6-RUNX1). Presence of MLL (KMT2A) Gene Rearrangement e.g. t(4;11) (q21;q23) and Philadelphia chromosome positivity t(9;22) results in poor prognosis.

MLL (KMT2A) Gene Rearrangement seen in 80% of Infant ALL and it has poor prognosis.⁵ t(12;21) (p13;q22) TEL/AML1 fusion observed in 25% of common ALL is associated with favorable prognosis. Presence of t(9;22)BCR-ABL1 fusion in precursor B ALL is associated with poor prognosis. Many other cytogenetic and molecular aberrations were observed in ALL. For example, recent studies have shown t(1; 19) (q23; p13) associated with the formation of TCF3-PBX1 fusion was associated with a poor prognosis in childhood B-ALL, although new anti-metabolic drugs used in chemotherapy increased patients survival due to appropriate therapeutic response.⁶ Several chromosomal translocations decrease therapeutic responses and increase mortality in patients. Studies showed t(9; 22) (q34, q11.2) translocation leads to BCR and ABL1 genes fusion, and ultimately leads to the formation of the Philadelphia chromosome (Ph).⁷ The resulting fusion gene BCR/ABL1, encodes for chimerical oncoprotein which leads to uncontrolled proliferation and reduced apoptosis. Therefore, the detection of t(9; 22) (q34, q11.2) BCR/ABL 1 translocation in ALL patients can increase the survival of patients before developing the disease by employing optimal therapeutic strategies.8

Genome-Wide Association Studies (GWAS) use techniques that are not widely available to define genetic abnormalities in the 20% of B-lineage ALL cases where routine testing is unrevealing, demonstrating changes associated with poor outcome like "Ph-like" ALL (10-15% of cases) with gene expression similar to Ph+ ALL and Increased CRLF2 (cytokine receptor like factor 2) expression, which may have an underlying CRLF2 mutation (5-7% of cases). Interestingly, increased CRLF2 expression is also seen in 50% of the patients with Down syndrome and ALL, where its prognostic value is uncertain.

Therefore, in this study, authors assessed the frequency of select molecular genetic abnormalities in children with B lineage ALL.

METHODS

Department of Pediatric Hematology, Institute of Child Health, Chennai, Tamil Nadu, India. Study design was hospital based Retrospective observational study. Study period was January 2018 to September 2019 (21 months).

Inclusion criteria

 All Children with confirmed diagnosis of B lineage Acute Lymphoblastic Leukemia with Genetic Molecular studies report were included.

Exclusion criteria

 Children with Leukemia other than B lineage Acute Lymphoblastic Leukemia, B ALL relapse cases and B ALL children without molecular studies were excluded.

Parameters studied

BCR/ABL 1 translocation assay, TEL /AML 1(ETV6//RUNX1) translocation assay, MLL (KMT2A) Gene Rearrangement assay.

Procedure

Case records of all confirmed ALL cases were analyzed. In those case sheets, all suspected Leukemia cases underwent Bone marrow aspirate. ALL was diagnosed by bone marrow morphology with significant blast count. After that Immune Phenotyping by Flowcytometry was done in either Bone marrow sample or if possible, in peripheral blood itself to identify the subtype of ALL. A volume of 5 mL of BM sample or Blood was collected from each patient in falcon tubes containing heparin and EDTA anticoagulants for performing cytogenetic and molecular genetic analyses, respectively.

Patients has received treatment based on ICiCLe protocol. This study was approved by the Local Ethics Committee. Molecular genetic analysis was done using Fluorescent in Situ Hybridization [FISH] technique. FISH technique was performed on BM or blood samples according to the guidelines of kit manufacturer for each patient.

Data collection and analysis

Data was collected from the case records of study period and recorded in the pro-forma and data was entered in Microsoft excel. This study was an epidemiological analysis. Descriptive data analysis was conducted using SPSS software.

Ethical consideration

No invasive procedure was involved. Only case records were analyzed. Confidentiality of the information was maintained. Consent was obtained from the parents.

RESULTS

Out of total 199 cases confirmed ALL 163(82%) were B lineage (Males 85(52%) and Females 78(48%)) (Table 1). 79(48%) cases of B lineage ALL (Males 37(46%) Females 42(54%)) underwent genetic molecular analysis by FISH method. The age group for 79 ALL cases ranged from 5months to 14 years Median age: 5 years (Males 6 months to 14 years Median age 5 years; Females 5 months to 12 years Median age 4 years). 6(8%) cases of infantile ALL were present (Males 1(17%); females 5(83%)).

Table 1: Immunophenotyping of acute leukaemia patients.

Sub Type of ALL	Male	Female	Total n=199
B lineage	85(52%)	78(48%)	163(82%)
T lineage	25(69%)	11(31%)	36(18%)

Cytogenetic and molecular analysis

Out of 79 cases of B lineage ALL, Translocation t(9;22) BCR/ABL1 was positive in 2(2.5%) cases (Male 1(50%); Female 1(50%)), 77(97.5%) cases (Males 36(47%); Females 41(53%)) were tested negative (Table 2).

Table 2: Frequency of t(9;22) BCR/ABL1 abnormality in B-Acute Lymphoblastic Leukemia patients.

Cytogenetics	Male n=37(46%)	Female n=42(54%)	Total n=79(100%)
t(9;22) Positive	1 (2.7%)	1(2.4%)	2(2.5%)
t(9;22) Negative	36 (97.3%)	41(97.6%)	77(97.5%)

Out of 79 cases of B lineage ALL, Translocation t(12,21) TEL/AML1 was positive 9(11%) cases (Male 3(33%); Female 6(67%)), and 70(89%) cases (Males 34(67%); Females 36(33%)) were tested negative (Table 3). Out of 79 cases of B lineage ALL, MLL Gene Rearrangement was seen in 6(7.6%) children. All the positive children were females 6(100%) (Table 4). Out of 79 cases of B lineage ALL, 6(7.6%) were Infantile ALL (Males 1(17%); Females 5(83%)). 4(67%) cases were positive

for MLL Gene arrangement, all of them were female children (Table 5).

Table 3: Frequency of t (12;21)
TEL/AML1(ETV6/RUNX1) abnormality in B acute lymphoblastic leukaemia.

Cytogenetics	Male n=37(46%)	Female n=42(54%)	Total n=79(100%)
t(12;21) Positive	3(8%)	6(14%)	9(11%)
t(12;21) Negative	34(91%)	36(86%)	70(91%)

Table 4: Frequency of MLL(KMT2A) gene rearrangement abnormality in B-Lineage acute lymphoblastic leukaemia.

Cytogenetics	Male n=37	Female n=42	Total n=79(100%)
MLL Positive	0(0%)	6(14%)	6(7.6%)
Negative	37(100%)	36(86%)	73(92.4%)

Table 5: Frequency of molecular abnormalities in Infantile B -Acute lymphoblastic leukemia.

Total n=6 (7.6%)	BCR/ABL1	TEL/AML1	MLL(KMT2A)
Male n=1 (17%)	0	0	0(0%)
Female n=5 (83%)	0	0	4(100%)
Total	0	0	4(67%)

Over all 17(22%) cases (Males 4(24%); Females 13(76%)) were positive for molecular abnormalities. In this study, t(12;21)TEL/AML1 was the highest frequency of molecular genetic abnormalities in patients with B-Lineage ALL. MLL(KMT2A) Gene Rearrangement was the frequent molecular abnormality observed in Infantile ALL.

DISCUSSION

ALL is a hematological malignancy develops as a result of the increased proliferation of lymphoid precursors and impairment of their differentiation. A series of genetic and molecular changes that are associated with certain clinical features cause this impairment. Studies showed the frequency of genetic and molecular changes in different parts of the world is different. Forestier et al, in 2000, showed that frequency of translocation t(9; 22) (q34; q11) was found in 2.2% of their study population. In It is comparable to this study, which recorded 2.5% positivity for t(9;22), which is least common compared to t(12;21) and MLL Gene Rearrangement.

Chopra et al, study showed that BCR/ABL1 was the frequently detected molecular abnormality in pediatric B-AL.¹¹ This is in contrast to this study, BCR/ABL1 was the least detected molecular abnormality even though

both studies are done in Indian children. Similarly a study by Arash et al, found translocation t(9;22) was the most common molecular abnormality in childhood B ALL.¹²

However, Andreasson et al, in 2000 showed that del (9p) was the most common chromosomal structural abnormality in children with preB ALL.¹³

In this study, abnormal t(12;21)TEL/AML1 was the highest percentage of molecular genetic abnormalities in patients with B lineage ALL(Table 3). These results are comparable to the to the results of De Braekeleer et al, study, which showed that t(12;21) (p13;q21) was the most frequent structural rearrangements in children with B ALL even though t(9;22) (q34;q11) was the most frequent structural rearrangements among adults with B-ALL.¹⁴

In contrast to this study, the low frequency of translocation t(12;21)TEL/AML1 among B ALL patients (Table 3) was observed by Arash et al, and Chebihi et al. 12,15 In this study MLL(KMT2A) Gene Rearrangement was the most commonly found molecular aberration in infantile B ALL which is comparable to the study done by Vijayanarasimha et al. 5

Few limitations of this study are, Karyotyping and translocation t(1;19) were not performed. Karyotyping might have helped to detect few other abnormalities like Hyper diploidy and Hypodiploidy which have prognostic value. Hyper diploidy (>50 chromosomes/cell) observed in 25% of children with B ALL children, associated with non-random trisomies X, 4, 6, 10, 14, 17, 18 and 21. Trisomy 4, 10 and 17 ['triple trisomy'] has been found to confer benefit. Extreme hypodiploidy with <44 chromosomes/cell confer poor prognosis observed in 1% of children with B lineage ALL.

In this study, t(1;19) (q23;p13) or TCF3-PBX1 was not tested in these children. Translocation t(1;19) is known to cause inferior survival outcome, usually it is observed in around 5% of children with B ALL.

CONCLUSION

t(12;21) TEL/AML1(ETV6/RUNX1) was the frequent molecular abnormality observed in B lineage ALL in south India and MLL(KMT2A) Gene Rearrangement was the frequent abnormality in Infantile B ALL. Genetic and molecular variations were different in comparison to other studies, authors concluded environmental, geographic, and population factors caused these differences among different populations.

This study has limitations since Karyotyping and translocation t(1;19) (q23;p13) were not performed. On the other hand, the evaluation of these molecular changes can be used as diagnostic and prognostic factors along with other clinical parameters in monitoring the patient and this hypothesis requires further studies in the future.

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REFERENCES

- 1. Horowitz NA, Akasha D, Rowe JM. Advances in the genetics of acute lymphoblastic leukemia in adults and the potential clinical implications. Expert rev Hematol. 2018 Oct 3;11(10):781-91.
- 2. Whitlock JA. Down syndrome and acute lymphoblastic leukaemia. Bri J Haematol. 2006 Dec;135(5):595-602.
- Hasle H. Pattern of malignant disorders in individuals with Down's syndrome. Lancet Oncol. 2001 Jul 1;2(7):429-36.
- 4. Mi JQ, Wang X, Yao Y, Lu HJ, Jiang XX, Zhou JF, et al. Newly diagnosed acute lymphoblastic leukemia in China (II): prognosis related to genetic abnormalities in a series of 1091 cases. Leukemia. 2012 Jul;26(7):1507.
- Vijayanarasimha D, Madhumathi DS, Kumari P, Naik J, Appaji L, Lakshmidevi V. The phenogenotypic characteristics of infantile acute leukemia in a regional cancer center from South India. J Appl Hematol. 2017 Apr 1;8(2):61.
- 6. Yiallouros DB, Henze G. Akute lymphoblastische Leukämie (ALL). Therapie. 2006;52006.
- Guo Y, Shan Q, Gong Y, Lin J, Yang X, Zhou R. Oridonin in combination with imatinib exerts synergetic anti-leukemia effect in Ph+ acute lymphoblastic leukemia cells in vitro by inhibiting activation of LYN/mTOR signaling pathway. Cancer Biol Thera. 2012 Nov 16;13(13):1244-54.
- 8. Jones LK, Saha V. Philadelphia positive acute lymphoblastic leukaemia of childhood. Br J Haematol 2005;130:489-500.
- 9. Schotte D, De Menezes RX, Akbari Moqadam F, Khankahdani LM, Lange-Turenhout E, Chen C, et al. MicroRNA characterize genetic diversity and drug resistance in pediatric acute lymphoblastic leukemia. Haematologica. 2011;96:703-11.
- Forestier E, Johansson B, Borgström G, Kerndrup G, Johannsson J, Heim S, NOPHO Leukemia Cytogenetic Study Group. Cytogenetic findings in a population-based series of 787 childhood acute lymphoblastic leukemias from the Nordic countries. Eur J Haematol. 2000 Mar;64(3):194-200.
- 11. Chopra A, Soni S, Verma D, Kumar D, Dwivedi R, Vishwanathan A, et al. Prevalence of common fusion transcripts in acute lymphoblastic leukemia: A report of 304 cases. Asia-Pacific J Clin Oncol. 2015 Dec;11(4):293-8.
- 12. Alqasi A, Tavakolifar Y, Rezaeeyan H, Saki N, Bagherpour S, Nasab MA, Cytogenetic and

- molecular assessment of childhood acute lymphoblastic leukemia patients from 2014 to 2017 in Ahvaz. Cli Cancer Invest J. 2019;8(1):28-32.
- Andreasson P, Höglund M, Békássy AN, Garwicz S, Heldrup J, Mitelman F, et al. Cytogenetic and FISH studies of a single center consecutive series of 152 childhood acute lymphoblastic leukemias. Eur J Haematol. 2000 Jul;65(1):40-51.
- De Braekeleer E, Basinko A, Douet-Guilbert N, Morel F, Le Bris MJ, Berthou C, et al. Cytogenetics in pre-B and B-cell acute lymphoblastic leukemia: a study of 208 patients diagnosed between 1981 and 2008. Cancer Gene Cyto. 2010 Jul 1;200(1):8-15.
- 15. Chebihi ZT, Belkhayat A, Chadli E, Hilal L, Skhoun H, Hessissen L, et al. Cytogenetic profile

- of Moroccan pediatric acute lymphoblastic leukemia: Analysis of 155 cases with a review of the literature. Clin Lymphoma Myeloma Leuk 2018;18:e241-8.
- 16. Paulsson K, Johansson B. High hyperdiploid childhood acute lymphoblastic leukemia. Genes, Chromo Cancer. 2009 Aug;48(8):637-60.

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