DETECTION OF THE THREE FUSION ONCOGENES OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA – EXPERIENCE IN A DEVELOPING COUNTRY, INDIA.

RACHEL PRIYANKA PULLA[1], TEENA KOSHY[2], SOLOMAN.F.D.PAUL[3], LATHA MAGATHA SNEHA[4], JULIUS XAVIER SCOTT[5].

1. PEDIATRIC RESIDENT, 2. SENIOR LECTURER OF HUMAN GENETICS, 3.PROFESSOR OF HUMAN GENETICS, 4. ASSISTANT PROFESSOR OF PAEDIATRIC HAEMATOONCOLOGY, 5. PROFESSOR/HEAD OF PEDIATRIC HAEMATOONCHOLOGY.

Sri Ramachandra Medical College and Research Institute, Porur, Chennai, India.

INTRODUCTION

- Acute Lymphoblastic Leukemia (ALL) is the most common childhood tumor, and although more than 80% of children are cured, relapsed ALL remains a leading cause of childhood morbidity and mortality.
- ALL is a heterogeneous disease and comprises of many different genetic subgroups as identified by various chromosomal and molecular abnormalities, with disparate clinical response to treatment regimen.
- This heterogeneity is likely to be due to genetic, racial and geographic variations that exist among different populations.

CONSEQUENCES OF NON-TARGETED TREATMENT

- Overly intensive treatment leads to
  - Development of secondary cancers
  - Reduction of IQ
- Insufficiently intensive treatment leads to
  - Relapse

TARGETED TREATMENT IS BASED ON GENETIC MARKERS

- The most common oncogenes found in leukemia patients are the **fusion genes**, which are formed as a result of different genetic abnormalities at the chromosomal level.
- The three major **risk stratifying** translocations in patients with ALL are

BCR-ABL - t(9;22)(q34;q11) | *TEL-AML* - t(12;21)(p13;q22) | *MLL* - 11q23 rearrangments

- Karyotyping, fluorescence in Situ hybridization (FISH) and real time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) are nowadays routinely used to detect genetic abnormalities.
- As these 3 tests provide information on similar anomalies it would be of interest to delineate the value of each test separately.

OBJECTIVES OF THE STUDY

- To determine the diagnostic accuracy of conventional karyotyping, FISH, and RT-PCR in childhood acute lymphoblastic leukemia.
- To determine the frequency of t(9;22)(q34;q11) (ABL/BCR) abnormalities using a combination of the above three techniques.
- To determine the frequency of t(12;21)(p13;q22) (TEL/AML1) abnormalities using a combination of the above three techniques.
- To determine the frequency of 11q23 rearrangments (MLL) rearrangements using a combination of the above three techniques.

SUBJECTS AND SAMPLES

- The present study comprised of PBL/BMA collected from 35 patients diagnosed with ALL (from the Division of Hemato-oncology, Sri Ramachandra Medical College)

Inclusion Criteria

- Ages of < one to fifteen years, with a confirmed diagnosis of ALL
- Patients with confirmed diagnosis of ALL sent for genetic testing

Exclusion Criteria

- Patients for which informed consent could not be obtained
- Patients diagnosed with leukemias/lymphomas other than ALL

METHODOLOGY

Cytogenetic studies

- *FISH analysis using probe manufacturers protocol*
- *Karyotyping using standard protocols*

Molecular genetic studies

- *RNA extraction using Trizol*
- *Synthesis of cDNA*
- *RT-PCR using standard protocols*

OVERVIEW OF RESULTS OF KARYOTYPING, FISH AND RT-PCR IN THE TESTED ABERRATIONS

Overview of results of karyotyping, Fish and RT-PCR in the Testes Aberration

	NO. of Cases	karyotyping	Fish	RT-PCR
BCR-ABL				
karyotyping+	1	NA	1/1 detected 0/4 not detected	1/1 detected 0/4 not detected
Fish+	1	1/1 detected 0/4 not detected		
PCR+	1			
TEL-AML1				
karyotyping+	0	NA	2 detected	2 detected
Fish+	2	0/2 detected 2/2 not detected	NA	1/2 detected 0/2 notdetected
PCR+	2	0/2 detected 2/2 not detected	2/2 detected 2/2 not detected	NA
MLL-AF4				
karyotyping+	0	NA	1 detected 0 not detected	1 detected 0 not detected
Fish+	1	0/1 detected 1/1 not detected	NA	1/1 detected 0/4 not detected
PCR+	1	0/1 detected 1/1 not detected	1/1 detected 0/1 not detected	NA

FISH indicates fluorescence in situ hybridization; PCR, polymerase chain reaction

RESULTS OF DIAGNOSTIC ACCURACY PARAMETERS OF KARYOTYPING, FISH, AND RT-PCR

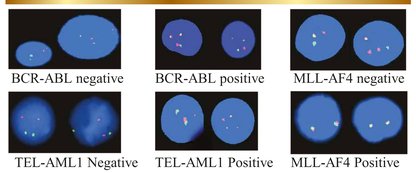
	Karyotyping	FISH	RT-PCR
t(9 ; 22)			
Sensitivity (95 % CI)	80 %	100 %	100 %
PV- (95 % CI)	100 %	100 %	100 %
LR- (95 % CI)	0.2	0	0
t(12 ; 21)			
Sensitivity (95 % CI)	6%	100 %	100 %
PV- (95 % CI)	87 %	100 %	100 %
LR- (95 % CI)	0.94	0	0
11 q23			
Sensitivity (95 % CI)	6%	100 %	30 %
PV- (95 % CI)	87 %	100 %	75 %
LR- (95 % CI)	0.94	0	0.7

FISH indicates fluorescence in situ hybridization; LR likelihood ratio of negative test; PV , negative predictive value; RT-PCR, real time quantitative reverse transcriptase polymerase chain reaction; 95% CI, 95% confidence interval.

FREQUENCY OF REARRANGEMENTS DETECTED BY 3 TECHNIQUES

Rearrangements	Frequency of Aberration
BCR-ABL	1 (3%)
TEL-AML	2 (6%)
MLL	1 (3%)
Total	11.4%

REPRESENTATIVE FISH IMAGES

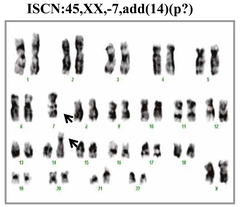


BCR-ABL negative | BCR-ABL positive | MLL-AF4 negative

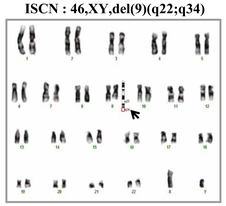
TEL-AML1 Negative | TEL-AML1 Positive | MLL-AF4 Positive

REPRESENTATIVE KARYOTYPE IMAGES FROM BONE MARROW ASPIRATE

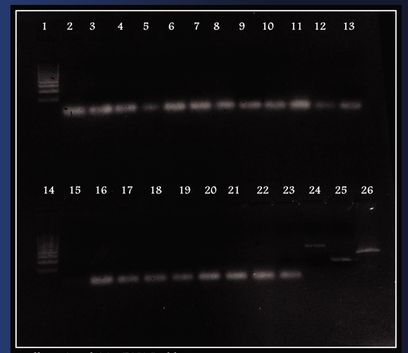
ISCN:45,XX,-7,add(14)(p?)



ISCN : 46,XY,del(9)(q22;q34)



A 3.5 % AGAROSE GEL WITH REPRESENTATIVE PCR PRODUCTS OF A MULTIPLEX PCR REACTION



well no .1 and 14 – DNA Ladder
well no. 2–13, 15–23 – Cases
well no. 23 positive control (MLL), 24 positive control (BCR-ABL), 25 positive control (TEL-AML)

DISCUSSION

- In this study the sensitivity of karyotyping, FISH, and RT-PCR-analysis for the detection of 3 specific chromosomal translocations t(12;21)(p13;q22), t(9;22)(q34;q11), and t(11q23) generally proved to be high.

  - The sensitivity of karyotyping in detecting the TEL-AML1 translocation and MLL rearrangements was found to be low (6%), as expected as theseare cytogenetically cryptic rearrangements. This is in line with reports in literature

  - Sensitivity of FISH for detection of BCR-ABL, TEL-AML1 fusions, and MLL-rearrangements was very high, which is in concordance to other studies

  - RT-PCR had a high sensitivity in detecting translocations causing the BCR-ABL and TEL-AML1, and MLL-AF4 fusion genes, which is in agreement with other studies.

- The frequencies of aberration obtained was found to be 3% for BCR-ABL, 6% for TEL-AML and 3% for MLL-AF4 This was slightly lower than reported in other studies. this could be possibly due to the small study population and the genetic susceptibility differences between different ethnicities.
- Karyotyping was an indispensable tool for discovering numerical, structural, and unexpected chromosomal aberrations.
- FISH and RT-PCR had additional value in certain anomalies which were cryptic or in those cases where karyotyping had failed.

CONCLUSION

- Karyotyping, FISH, and RT-PCR are powerful tools for the detection of the major chromosomal abnormalities in childhood ALL, although each method has its limitation.
- The complementary use of the techniques in ALL diagnostics, in combination with minimal residual disease detection, will deliver the best available treatment to an individual child.

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