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Cytogenetic analysis using Fluorescence in situ Hybridization (FISH) of Acute Lymphoblastic Leukemia

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Abstract
In pediatric acute lymphoblastic leukemia (ALL), chromosomal abnormalities are highly significant prognostic factors. In the present study peripheral blood or bone marrow samples of 60 children between age group of 1-17 diagnosed with ALL were analyzed for chromosomal abnormalities with fluorescence in situ hybridization (FISH) using locus specific probes to detect cryptic TEL/AML1 t (12:21) (p13-q22), BCR/ABL t (9;22) (q34;q11), TCF3- PBX1T(1:19)(q23;p13) and MLL rearrangement for 11q23. In the present study out of 60 cases 49 cases (81.66%) were normal and in 11 cases (18.33%) translocations were detected. Translocation t (12;21) was found in 7 cases and translocation t(9;22) was found in 4 cases. Any translocation was not detected in the age group of 1 to 9 years. 30.76% male cases and 13.04% female cases found to have translocations. Results showed that FISH improved the sensitivity and accurate identification of prognostic markers in pediatric ALL patients.

Keywords: Cytogenetics, FISH, All, acute leukemia, structural rearrangements

Introduction
Acute lymphoblastic leukemia (ALL) is the most common childhood cancer and accounts for about 25% of cancer diagnoses among children under 15 years of age (Howlader *et al*., 2013) [5]. Acute lymphoblastic leukemia (ALL) is characterized by many recurrent cytogenetic abnormalities. In ALL, cytogenetics is now routinely used as a diagnostic procedure (Mrozek *et al*., 2009) [6]. A systematic analysis of several thousands of patients has revealed abnormalities with major prognostic value, some predicting a very poor outcome, others correlated with extremely high chances of long-term survival. In conventional cytogenetics, however, these chromosomal abnormalities may not be detected due to the absence of metaphase within the leukemic clone, poor chromosomal morphology and cytogenetically invisible abnormalities. Consequently, complementary techniques are required to determine the prognosis of each patient. FISH appears to be the best method because of its specificity, rapidity, and feasibility (Gonzales and Mikhail, 2017) [3].
FISH can detect all genetic abnormalities that influence prognosis directly on cytogenetic preparations. Further, it is possible to obtain this information within 24 hours, allowing for patient-specific treatment. Using such systematic analyses, it is possible to define the exact prognostic impact of each abnormality, allowing risk-adjusted strategies to be proposed for ALL patients (HervÉ Avet-loiseau, 1999) [4].
As survival improved, it became apparent that some of these chromosome changes had major prognostic significance: t (12; 21) are associated with long disease free survival, but t(1;19), t(9;22) and llq23 rearrangements correlate with a poor outcome (Bhojwani *et al*., 2009) [2]. As a result of this prognostic significance, these four specific abnormalities must be detected accurately and rapidly. In order to reliably analyze ALL patients, we used fluorescence in situ hybridization (FISH) in the present study. 60 pediatric patients under the age of 17 were studied by FISH for the detection of chromosomal abnormalities. Specific probes were selected that allow direct detection of structural rearrangements during interphase. Through this technique, it is possible to detect chromosomal gains and losses without requiring the acquisition of metaphases.

Materials and Method
Patient Samples- Peripheral blood or bone marrow sample was obtained at diagnosis from 60 ALL pediatric patients in the age group of 0 to 17 years during January 2014 to December 2014 to the Oncquest Laboratories, India.

Chemicals: MQ, PBS, Fixative, Pepsin (0.25%), 1N HCl, SOL A, B, C and D, Probes, Formaldehyde, SSC, NP40.

Instruments: Electronic weighing balance, Centrifuge, Water bath, Drying oven, Diamond marker, Vortex, Light microscope, Thermobrite, Fluorescent microscope, CCD camera, Computer.

Equipments: Sodium heparin vacutainers, Sterile culture tubes, Micropipette and tips, Droppers, Glass slide, Coupling jars, Coverslips.

Reagent preparation
Sol A: 49 ml MQ, 500 µl 1N HCl and 225 µl pepsin (2.5%) were added and kept at 37 °C.
Stock was prepared by mixing 0.25 gm in 10 ml saline and pH was set at 1.2 to 2.
Sol B: 49 ml MQ (PBS 1X) and 1.34 ml formaldehyde were added and kept at 4 °C.
Sol C: 49 ml MQ, 1 ml SSC (20X) and 150 µl NP40 were added and kept at 73 °C.
Sol D: 45 ml MQ, 5 ml SSC (20X) and 50 µl NP40 were added and kept at room temperature (RT).
Firstly, TLC of all the samples were taken. 10 ml of 1X PBS in a pre-labeled centrifuge tube was added. 60-1000 µl (according to TLC) of the sample was added. Mixed gently and centrifuged at 3000 rpm for 5 mins at R.T. supernatant was discarded and resuspended the pellet well and washed the pellet for twice. On the day of dropping the suspension was washed twice.

Slide preparation
The diluted suspension was dropped on prelabeled washed glass slides. The slides were allowed to dry and observed under microscope for even cell distribution. Slides were placed at 70 ºC for 1 hr for aging.

Pre-Hybridization
The slides were aged and then slides were dehydrated with 70%, 85%, 100% ethanol for 3 min each. The slides were kept in SOL A for 10 mins at 37 ºC. Rinsed twice in MQ water and once in 1X PBS. The slides were kept in SOL B for 10 min at 4 ºC. Slides were dehydrated with 70%, 85% and 100% ethanol for 3 min each, air dried.

Hybridization
Thaw the appropriate probe to room temperature. Spin down the contents in the tube and then a 2.5µl probe was added in dark. Sealed with the coverslip and the sample DNA and the probe DNA are then co-denatured in thermobrite at 80 ºC and allowed to re-anneal for 17 hours at 37 ºC.

Post hybridization washings
Coverslip was removed and kept the slides in SOL C at 73 ºC for 3 mins. Immediately slides were transferred into SOL D for 3 mins. Slides were removed from SOL D and allowed to dry. The slides were washed to remove excess unbound probe, and counterstained with 4',6-Diamidino-2-phenylindole (DAPI) and Antifade (2:1).

Analysis
Analysis of FISH specimens was done by fluorescence microscopy. Interphase cells were scored, generally 200 cells were counted and scored (Shaffer and Gowan, 2013; Barch *et al*., 1997) [7, 1].

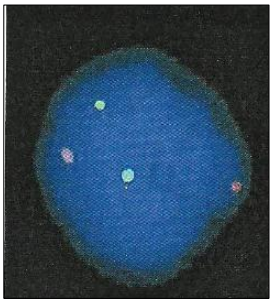
Software
We have used Cytovision software for analyzing the results obtained.

Results and Discussion
In the present study, a total of 60 cases (34 male and 26 female) of pediatric patients under the age of 17 were studied by FISH for the detection of chromosomal abnormalities.

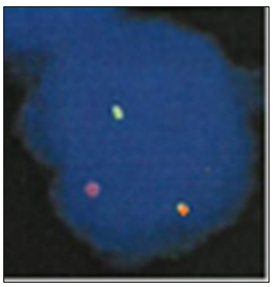
Result Interpretation for FISH
1. BCR –ABL

t(9;22) (q34;q11.2), BCR- (Ch- 22) Green, ABL- (ch- 9) Red

Negative cases – 2 green and 2 Orange signal
Positive Cases- 1 green 1 orange and 1 Fusion (yellow) or 2 Fusion signals.

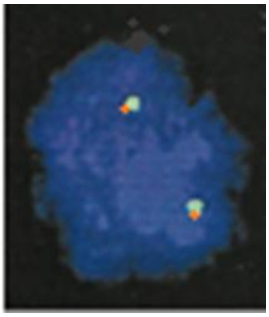


Negative -2g2o

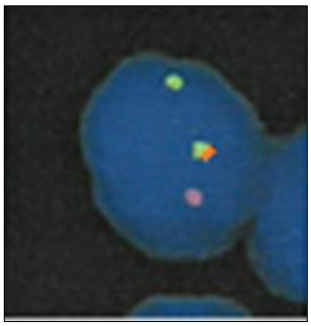


Positive – 1g1o1F

MLL Gene
11q23 break apart fusion gene.
Negative cases- 2 yellow signals.
Positive Cases – 1 orange 1 green and 1 fusion signal.



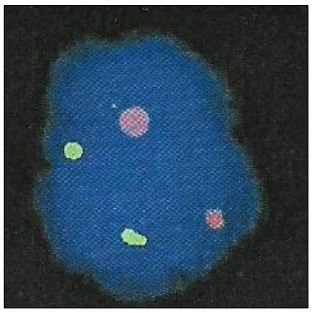
Negative -2f



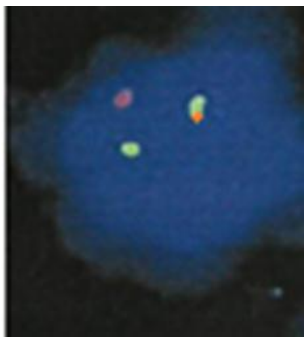
Positive – 1g1o1F

TEL – AML

t(12;21)(p13;q22), Tel- (ch 12) - green, AML- (ch 21)- orange
Negative cases – 2 green and 2 Orange signals
Positive Cases- 1 green 1 orange and 1 Fusion (yellow) or 2 Fusion signals.



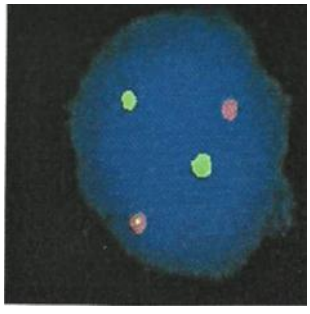
Negative- 2g2o



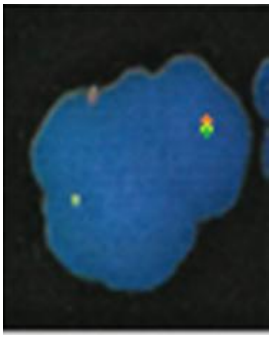
Positive- 1g1o1F

TCF3- PBX1

T(1:19)(q23;p13), PBX1(ch- 1)- orange, TCF3(ch-19)- Green
Negative cases – 2 green and 2 Orange signals
Positive Cases- 1 green 1 orange and 1 Fusion (yellow) or 2 Fusion signals



Negative- 2g2o



Positive- 1g1o1F

In the present study out of 60 cases 49 cases (81.66%) were normal and in 11 cases (18.33%) translocations were detected. Translocation t (12;21) was found in 7 cases and translocation t(9;22) was found in 4 cases. Any translocation was not detected in the age group of 1 to 9 years (Table 1; Fig 1). 30.76% male cases and 13.04% female cases found to have translocations (Table 2; Fig 2). We showed that this FISH-based approach was complementary to conventional cytogenetics, and should be systematically used in ALL at diagnosis.

Table 1: Age wise distribution of translocations by FISH

Age group	Total number of cases	Normal	t(12;21)	t(9;22)
0-1	4	4	-	-
1-9	44	36	6	2
10-13	4	2	1	1
14-17	8	7	-	1

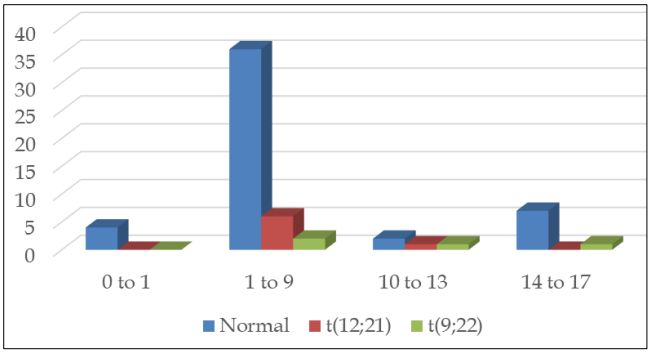


Fig 1: Age wise distribution of translocations by FISH

Table 2: Gender wise distribution of translocations by FISH

Gender	Normal	t(12;21)	t(9;22)
Male	26	6	2
Female	23	1	2

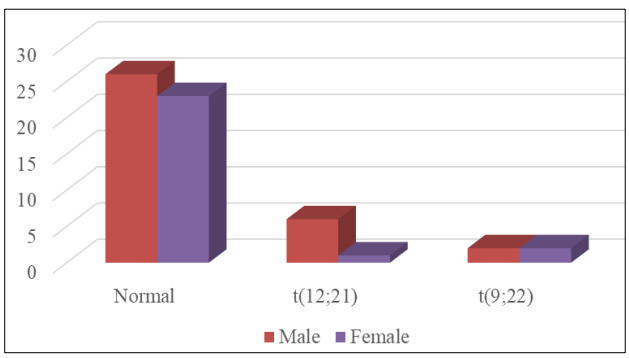


Fig 2: Gender wise distribution of translocations by FISH

Conclusion

It was concluded that in cytogenetic laboratories, it is recommended to routinely apply advanced molecular methods to detect cryptic abnormalities. By studying pediatric ALL cytogenetics in this study, we continue to strengthen and expand our understanding of this disease. A prognostic evaluation of cytogenetic abnormalities for ALL patients is also recommended in the future. Further, a study with a larger number of patients is needed to determine whether gene rearrangements are associated with outcomes so that the cause of leukemia can be better understood.

Future scope

It will provide useful diagnostic and prognostic information on a variety of common, rare, and novel chromosomal abnormalities in patients with hematological disorders.

Conflict of Interest: On behalf of all authors, the corresponding author states that there is no confict of interest.

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