RESEARCH ARTICLE

Combined Study of Cytogenetics and Fluorescence in Situ Hybridization (FISH) Analysis in Childhood Acute Lymphoblastic Leukemia (ALL) in a Tertiary Cancer Centre in South India

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Abstract

    FISH is one of the most sensitive molecular methods to detect genetic abnormalities with DNA probes. When cytogenetic studies are normal or insufficient, FISH may detect cryptic rearrangements, rare or slowly proliferative abnormal populations in non-mitotic cells. We cytogenetically evaluated 70 childhood ALL - 67.1% were found to have an abnormal karyotype. The 23 patients (32.9%) with a normal karyotype were analyzed by FISH applying two probes; TEL/AML1 and MYB which detect cryptic rearrangements of t(12;21)(p13;q22) and deletion of (6q) respectively, associated with a good prognosis. Out of 23 patients, one was positive for t(12;21)(p13;q22) (4.3%). None of our patients were positive for MYB del(6q). Two patients showed an extra signal for MYB on chromosomes other than 6 (8.6 %) indicating amplification or duplication. Findings were compared with the available literature. Our study clearly indicated the integrated FISH screening method to increase the abnormality detection rate in a narrow range. FISH is less useful for diagnostic study of patients with suspected del(6q) but it helps in detecting known cryptic rearrangements as well as identification of new abnormalities(translocation , duplication and amplification) at the gene level.

Keywords: Acute lymphoblastic leukemia - cytogenetics - fluorescent in situ hybridization - MYB - TEL/ AML1

Introduction

    Acute lymphoblastic leukemia (ALL) is the most common leukemia in children, accounting for 25% of all pediatric malignancies (Hee et al., 2005). The peak incidence is between 2 to 5 years of age and approximately 90% contain clonal chromosome abnormalities at diagnosis (Sandoval et al., 2000; Riesch et al., 2001). Specific genetic abnormalities have been useful in diagnosis and defining prognostically important patient subgroups (Swolin et al., 2000, Chai et al., 2007). Although cytogenetic analysis by G- banding is a powerful tool for assessment of acquired chromosomal changes, the submicroscopic or cryptic rearrangements affecting regions smaller than a chromosomal band can also be extremely difficult to detect. Such limitations of cytogenetics can be eliminated by molecular techniques (Altaf et al., 2000). Fluorescent In Situ Hybridization (FISH) is one of the most sensitive molecular methods used to detect genetic abnormalities (Sundarashan et al., 2002). FISH can be easily performed on specimens prepared for cytogenetic studies, so application of FISH with normal cytogenetic results may represent an efficient and cost effective strategy to maximize the information obtained from clinical specimen. Hence, cytogenetics and FISH techniques are complementary in their sensitivity and specificity. We present the results of cytogenetics and FISH using probes for TEL/AML1 and MYB deletion to estimate the incidence of abnormalities involving above genes in childhood ALL patients in Kidwai Memorial Institute of Oncology, a centre for cancer care and treatment from India.

Materials and Methods

    Heparanized bone marrow aspirate was collected from seventy childhood ALL patients aged between 10 month to 14 years during 2009 to 2011 from pediatric oncology department (according to medical procedures) and processed for G- banding and FISH analysis.
Cytogenetics
    Bone marrow samples were cultured in RPMI 1640 medium (Gibco- Invitrogen, USA) containing 15% fetal Bovine serum for 24 and 48 hours at 37°C. After incubation, the cells were exposed to colcemid (Gibco, $0.10 \mu g/ml$) for 30 minutes, followed by hypotonic

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treatment (0.075 M KCl) for 20 minutes. Chromosomes were treated with Trypsin and stained with Giemsa (GTG-banding). Karyotypes were analyzed and interpreted according to the International System for Human Cytogenetic Nomenclature (Shaffer et al., 2009).

FISH

Air dried slides prepared from 23 bone marrow samples of cytogenetically normal karyotype patients washed in 2 x (Saline- Sodium Citrate buffer) for 2 minutes at room temperature followed by dehydration in an ethanol series (75%, 85% & 100%), each for 2 minutes and then allowed to dry. 10μl of probe mixture of TEL/ AML translocation and MYB del(6) (Cytocell aquarius- UK) were spotted on the cell sample and carefully coverslip was placed on the probe area and sealed the edge with rubber solution glue and allowed to dry completely. The slides were kept for denaturation, followed by 2 x SSC, 0.05% Tween-20. DAPI antifade (10μl) applied on drained slides covered with a coverslip and allowed the color to develop in the dark for 10 minutes. Slides were viewed with the use of appropriate filters and FISH software (Zeis Axiophate fluorescence microscope). Minimum 200 cells were scored for signals, whereas available metaphases were also captured.

As per kit insert, in the normal cell, TEL/ AML translocation Probe will appear as discrete red and green spots, one for each homologue. In MYB probe is there should be two red and two green signals in normal, whilst a detected cell has only shows one red signal and two green controls.

Results

Cytogenetics was performed for 70 childhood ALL patients. The male- female ratio was 2.68 and median age at diagnosis was 7.59 years. Out of 70 patients, 47 (67.1%) were found to have abnormal karyotype and rest 23 (32.9%) were normal. Seven patients (14.8%) had hyperdiploidy ranging from 47 to 59 chromosomes. The most common translocation were t(9;22)(q34;q11) in 6 patients (12.7%), followed by t(1;19)(q23;p13) in 5 (10.6%), t(4;11)(q21;q23) in 3 (6.3%) and t(4;12)(q21;p13) in 2 patients (4.2%). del(6)(q21) in 17(36.1%), del(9) in 4 (8.5%), del(5)(q13) in 2(4.2%) and del(12)(p12) in 1 (2.1%) patient were observed .

FISH was performed on 23 patients with normal karyotype which were proved to be ALL by clinical and hematological findings. Out of 23, one patient was positive to t(12;21)(p13;q22) for fusion gene TEL/ AML1(4.3%). The signal pattern is one green (native TEL) and red (native AML1) and two yellow for fusion of TEL and AML1 genes (Figure 1B). Rests of the patients showed two green and two red signals indicating absence of this specific rearrangement (Figure 1A). Using other probe, all 23 patients also showed two red and two green signals indicating the absence of MYB- del(6q) (Figure 2A). Two patients showed two green and three red signals, indicating two 6 chromosomes intact with MYB gene, and third red signal, the additional copy of MYB was seen on chromosome other than 6 (Figure 2B).
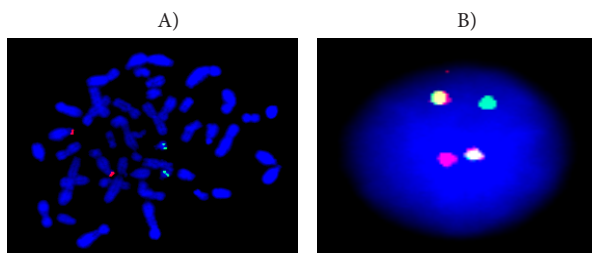
Figure 1. FISH Result of t(12;21). A) Metaphase of a normal cell with 2green signals indicates chromosome 21 (AML1) and 2red indicates chromosome 12 (TEL). B) Dual-color DNA probe showing the green and red signals of the normal chromosomes 12 & 21 with yellow / white signals, indicates that two region of TEL gene on chromosome 12 and AML1 gene on chromosome 21 are fused.
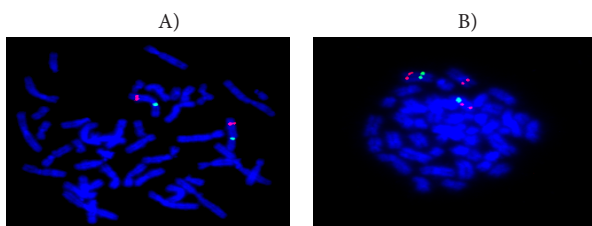

Figure 2. FISH Result of MYB Deletion. A) Metaphase showing 2 red (MYB) and 2 green (centromere of chromosome 6) of a normal cell. B) Metaphase showing an extra red signal of MYB.

Discussion

In present study diagnostically and prognostically important chromosomal abnormalities were detected in 67.1% of patients by Cytogenetics. The rate of abnormality increased to 71.4% applying FISH. High incidence of del(6)(q) and hyperdiploidy was followed by t(9;22)(q34;q11) and t(1;19)(q23;p13), indicating good prognostic cytogenetic markers like del(6)(q) and hyperdiploidy as more in this population. The t(12;21)(p13;q22) is a cryptic rearrangement which fuses the TEL gene on chromosome 12(p13) and the AML1 gene on chromosome 21(q22) and is difficult to detect by cytogenetics. The t(12;21)(p13;q12) is frequently associated with childhood B lineage ALL and it has a favourable prognosis with cure span in ≥90% especially if they have other favourable risk factors. Its frequency has been found to range from 14-25% by molecular techniques (Settin et al., 2007). In our study we detected t(12;21)(p13;q22) with a frequency of 4.34%. The geographical variation in the incidence of TEL/AML1 fusion has been suggested with frequency of 25% reported in the United States, France 15%, Germany and Italy 14% and the lower frequency of 13% in UK, Korea and Spain in B-lineage ALL (Gafini et al., 2002). The difference in frequency could be due to the patients' inclusion criteria. The association of t(12;21)(p13;q22) along with the other low risk features like younger age group, lower WBC count and pre-B phenotype raises the issue of the clinical significance of TEL/AML1 fusion as an independent favourable prognostic feature (Kyoun et al., 2001). Therefore, it appears that apparent prognostic significance of TEL/AML1 should be investigated further to evaluate whether this translocation alone or in association with other good prognostic features turn out

to be a strong predictor for prognosis.

Literature reveals varied various breakpoints in chromosome (6)(q15-27) up to 4.1-13% in patients of ALL (Borowitz et al., 2008). Frequently involved breakpoints are (6)(q15), (6)(q21-23) and interstitial deletion are also common in both B lineage and T lineage. Overall the breakpoints occur predominantly in (6)(q21). These deletions are mainly reported to be terminal which is difficult to estimate by cytogenetic analysis because of over condensation of chromosomes or too terminal deletions are beyond the sensitivity of the technique. The (6q) abnormality was reported to be a good prognostic indicator. None of our patients have del(6q), but in 2 patients (8.69%) an extra red signal of MYB was detected which leads to the assumption that the presence of MYB gene in the form of duplication or amplification was seen in a chromosome other than 6. Clinical relevance of this additional copy of MYB gene is not clear at present in these two patients.

Our study clearly indicates integrated FISH screening method, increased the abnormality detection rate in a narrow range. Application of FISH technique is not only helping in detecting known cryptic rearrangements, but also in identifying the gene expression of the respective genes. Further, the study with a larger number of patients would be necessary to know the relationship between each gene rearrangement and outcome to provide a better understanding of leukemiogenesis by new gene rearrangements identified in this study.

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