INTRODUCTION

Acute leukemia is characterized by the uncontrolled multiplication of undifferentiated hematopoietic precursors in blood, bone marrow, and lymphoid organs. It can originate from myeloid (acute myeloid leukemia [AML]) or lymphoid (acute lymphoid leukemia [ALL]) progenitor cells, with the latter having either T-cell or B-cell lineage origins (T-ALL or B-ALL). ALL is a neoplastic disease that results from a somatic mutation in a single lymphoid progenitor cell at one of the several discrete stages of development. In leukemia, an intramolecular fusion gene is formed which results in a protein with altered properties. ALL is the most common malignancy in children [1]. It accounts for 25% of all childhood cancers and approximately 75% of all cases of childhood leukemia [2].

The cure rate for ALL is >80% in children and approximately 35% in adults. Remarkable advances have been made over the past 15 years in the treatment of ALL, and the understanding of its pathophysiology and several important biomarkers have been established.

Pediatric ALL is often cited as one of the true success stories of modern medicine. The cure rates have improved from virtually zero, before the advent of modern chemotherapy and radiotherapy (in the 1950s), to the current overall event-free survival rates of approximately 80% [2]. This success and exemplary progress is largely due to the identification of various biomarkers responsible for good and poor prognosis, diagnosis, and improvement in the understanding of how to combine and use the chemotherapeutic agents effectively with minimum toxicity in supportive care.

Despite advances in the treatment, 20–30% of children with ALL in whom remission is achieved after initial induction chemotherapy, subsequently relapse. An array of clinical and lymphoblastic biological features have been identified as prognostically significant in childhood ALL, including age, presenting leukocyte count, immunophenotype, chromosomal abnormalities, the presence of overt central nervous system leukemia, and the rapidity with which the patients demonstrate a response to initial induction chemotherapy [3].

METHODS

Study design

The study was conducted at the Department of Molecular Biology and Transplant Immunology, Indraprastha Apollo Hospitals, New Delhi. A total of 144 newly diagnosed ALL patients were included in the study with their written informed consent. The clinics and other details were taken (Table 1). These patients were further classified on the basis of risk stratification, i.e., low-risk and high-risk patients on the basis of age, sex, platelets, and WBC count at the time of diagnosis as per the criteria of the National Cancer Institute, USA (Table 2). Immunophenotyping was done to further categorize these patients. Karyotyping was done as a routine investigation in the patients. Fluorescence in situ hybridization (FISH) was performed on Pre-B-ALL patients wherever possible. Five years survival analysis was also done in follow-up patient (Fig. 1a).

Cytogenetics studies and karyotype analysis

Heparinized bone marrow samples obtained at the time of diagnosis were processed and cultured for 24 h/48 h in RPMI 1640 medium (Caisson Labs, cat. no. RPMI-012P) and 20% fetal bovine serum is
ETV6/RUNX1 28 (23.7) ≤50,000 [116] 112 (78.1) 3.5:1 144
46 (31.6) 57 (38.6) >50,000 [28] 36 (26) 51 (36) 26 (18.4)
10–18 [36] ≤50×10
14 (9.7) 55 (33.3) 72 (50) 2 (1.7)
>50×10
Table 1: Clinical characteristics of the study patients

| Total patients (n) | 144 |
|-------------------|-----|
| Male (%)          | 112 (78.1) |
| Female (%)        | 32 (21.9) |
| Sex ratio         | 3.5:1 |
| Age (%)           | |
| 0–5 years         | 57 (38.6) |
| 5–10 years        | 51 (36) |
| 10–16 years       | 36 (26) |
| TLC (%/mm³)       | |
| <4000/µL          | 55 (33.3) |
| 4000–2000/µL      | 35 (24.6) |
| 20000–50000/µL    | 26 (18.4) |
| >50000/µL         | 28 (23.7) |
| Platelet count (%)| |
| <30×10¹²/L        | 26 (18.4) |
| 30–50×10¹²/L      | 72 (50) |
| >50×10¹²/L        | 46 (31.6) |
| Immunophenotype (%)| |
| Pre B-ALL         | 128 (88.6) |
| B-ALL             | 2 (1.7) |
| T-ALL             | 14 (9.7) |

B-ALL: B-cell acute lymphoblastic leukemia, T-ALL: T cell acute lymphoblastic leukemia, TLC: Total leukocyte count

Table 2: Risk stratification of patients at the time of diagnosis as per NCI criteria

| Risk group | Sex/n | Age (Years) | TLC (/µm³) | Platelets (L/µL) |
|------------|-------|-------------|------------|------------------|
| Low        | Females/32 | 0–9 [108] | ≤50,000 [116] | ≤50×10⁸ [98] |
| High       | Males/112 | 10–18 [36] | >50,000 [28] | >50×10⁸ [46] |

Risk stratification among patients on the basis of age, sex, TLC, and platelet counts. TLC: Total leukocyte count

added (GIBCO cat. no. 10270), COLCEMID (0.05 µg/ml) (Biological Industries 10 µg/ml cat. no. 12004-1D) was added for the past 60 min of culture, followed by hypotonic treatment with a 0.075-M KCl solution and a final fixation in methanol/acetic acid (3:1). Chromosomes were G-banded for identification whenever possible, at least, 20 metaphases were analyzed according to the International System for Cytogenetic Human Nomenclature, 2016.

FISH

FISH is a technique that involves the precise annealing of a single-stranded fluorescently labeled DNA probe to the complementary target sequences. The hybridization of the probe with the cellular DNA site is visible by direct detection using fluorescence microscopy. The interphase cells obtained from bone marrow specimens are processed for the FISH assay for the cryptic 12; 21 translocations (ETV6/RUNX1 or, historically, TEL/AML1) has applications in the diagnosis and monitoring of ALL. The 12; 21 cryptic translocation cannot be seen on G-banded metaphases; therefore, FISH and other molecular methods are needed to detect this rearrangement.

Survival analysis

Analysis of disease outcome was examined as overall survival (OS). The OS was measured from the date of initial diagnosis of ALL to the date of death from any cause or date of the last contact using the Kaplan–Meier method which is a nonparametric (actuarial) technique for estimating time-related events (the survivorship function) (Fig. 1a).

RESULTS

The clinical and laboratory features of pediatric ALL patients at diagnosis are summarized in Table 1. The age of the study subjects varied between 1.5 and 15.8 years. The mean and median age were 7.2 (±4.1) and 6.5 years, respectively. The gender distribution of the study subjects revealed that there were 112 male (78.1%) and 32 female (21.9%). The total leukocyte count (TLC) of the patients at the time of diagnosis ranged between 600 and 7 lakhs/cumm. The mean and the median TLC were 39,000/cumm and 8300/cumm, respectively. The TLC diagnosis was <4000, 4000–20,000, 20,000–50,000, and >50,000 in 55 (33.3%), 35 (24.6%), 26 (16.4%), and 20 (13.7%) patients, respectively. The platelet count of the patients at the time of diagnosis was <30×10⁸/L in 26 (18.4%) patients, 30×10⁸–50×10⁸/L in 72 (50%) patients, and more than 50×10⁸/L in 46 (31.6%) patients. The immunophenotyping results revealed Pre B-ALL profile in 128 (88.6%), B-cell ALL in 2 (1.7%), and T-cell ALL in 14 (9.7%) patients.

Karyotype analysis (Fig. 1b)

Out of the 144 patients, karyotype results were available in 131 (91%) patients and poor morphology in the remaining 13 (9%) patients. These patients were further divided according to cytogenetics abnormalities, i.e., numerical chromosome abnormalities (ploidy status) and structural abnormalities.

On the basis of ploidy, the patients were divided as diploid in 91 (69.4%) patients; pseudodiploid in 15 (11.45%) patients; hyperdiploid (>47–92 chromosome) in 15 (11.45%) patients, hyperdiploid with additional change in 3 (2.29%) patients, hypodiploid (<46 chromosome) in 4 (3.05%) patients, and hypodiploid with additional change in 3 (2.29%) patients. Trisomy of 21 chromosomes was seen in 8 patients and trisomy of chromosome 8 was seen in 4 patients. Other trisomies observed, including those of sex chromosome and their frequencies are depicted in Table 3.

Thirty-five (26.7%) structural abnormalities were observed in group of patients that included 9 (25.7%) translocations, 2 (5.71%) inversions, 11 (31.42%) deletions, 2 (5.71%) isochromsome,
Among the translocations, the most common translocation was t(9;22) in 5 (14.28%) patients. Deletions of chromosome 5, 7, and 9 were the most common deletions observed (Table 3).

**Karyotype and fish analysis**

For 30 pre-B-ALL patients, it was possible to perform both karyotype and FISH panel for all three probes, namely, TEL/AML1 t(12;21) (p13-q22), BCR-ABL t(9;22) and MLL, as per the clinicians’ choice.

Both karyotype and FISH analysis were negative for ETV6-RUNX1 gene in 17 (56.66%) out of 30 patients (Tables 4 and 5). ETV6-RUNX1 gene fusion was positive in 13 (43.3%) patients either by karyotype and/or by FISH analysis. Both karyotype and FISH analysis were positive for ETV6-RUNX1 gene in 8 (26.66%) out of 30 patients. In addition, 4 (13.33%) patients were found positive for ETV6-RUNX1 fusion gene by FISH analysis only, indicating cryptic translocation. Out of these, 13 (43.3%) patients positive for ETV6-RUNX1 gene, variant signals (one fusion) were observed in 3 (10%) patients. Hyperdiploidy was observed in 8 (26.66%) ETV6-RUNX1 fusion gene-positive patients which involved ETV6 in 6 (75%) patients and RUNX1 in 2 (25%) patients (Fig. 1c).

For 30 patients, it was possible to perform both karyotype and FISH panel for BCR-ABL gene. Both karyotype and FISH analysis were negative for BCR-ABL gene in 25 (83.33%) out of 30 patients. Both karyotype
and FISH analysis were positive for BCR-ABL gene in 5 (16.66%) out of 30 patients. Out of these five patients positive for BCR-ABL fusion gene, variant signals (one fusion) were observed in 1 (20%) patient. Hyperdiploidy was also observed in 1 (3.33%) BCR-ABL fusion gene-positive patient that involved the BCR (9q34) gene.

For 30 patients, it was possible to perform both karyotype and FISH analysis for MLL gene. Both karyotype and FISH analysis were negative for MLL gene in 26 (86.66%) out of 30 patients. MLL rearrangements were seen in 4 (13.3%) patients either by karyotype and/or by FISH analysis. Both karyotype and FISH analysis were positive for MLL gene in 2 (50%) out of 30 patients. In addition, 2 (50%) patients were positive for MLL gene rearrangement in FISH analysis only, indicating cryptic rearrangement. Hyperdiploidy and hypodiploidy were observed in 1 (3.33%) patient each of MLL rearrangement.

Survival analysis
Out of 144 patients, five-year survival data available for 70 patients were analyzed using Prism 7 software. A patient diagnosed with hyperploidy had better overall survival than patient with hypoploidy (Fig. 1a).

DISCUSSION
Acute leukemia is characterized by the uncontrolled clonal proliferation of hematopoietic precursor cells coupled with aberrant or arrested differentiation. ALL is the most common cancer diagnosed in children and represents 22% of cancer diagnoses among children younger than 15 years.

Numerous clinical and laboratory findings, including prognostic factors such as age, gender, cell count, pathophysiological, and cytogenetics at the time of diagnosis helps in determining the intensity and severity of disease and to predicts the best clinical outcome.

Demographic and clinical findings
In the present study, the age of the study subjects varied between 1.5 and 15.8 years with a mean and the median age of 7.2 (4±1.9) and 6.5 years, respectively. Advani et al. [4] reported a median age of 8.8 years in their study. The majority of patients (74.6%) in our study population were below 10 years of age indicating a younger study population. More so, a larger number of patients (38.6%) were <5 years of age. Our results are concurrent with Advani et al. [4]. The authors reported 60% and 57% patients, respectively, aged between 2 and 9 years [5,6]. Further reports by Wessel et al. and Silverman et al. have also supported our findings [7-8].

The gender distribution of the study subjects revealed that there were 112 male (78.1%) and 32 female (21.9%) with a male to female ratio of 3.5:1. A male to female ratio of 2.9:1, 2.6:1, and 2.14:1 was reported previously [4]. This distorted sex ratio is not uncommon from studies in India [4-6].

In the laboratory findings, the TLC ranged between 600 and 7 lakhs/L. The mean and the median TLC were 39,000/cumm and 8300/cumm, respectively. A mean TLC of 38.8 and 62.7×10⁹/L in two different population from South Africa, respectively [8]. In contrast, Silverman et al. reported a mean WBC count of 9.8×10⁹/L in 1255 patients [7]. In our study, the TLC was >50.0×10⁹/L in 28 (23.7%) patients. In three separate studies, it was observed that 26–30.9% of their patients had TLC >50.0×10⁹/L [4-6]. These findings are in concordance with our observation. However, Shanta et al. had reported TLC >60.0×10⁹/L in 60% of patients in their series previously [9].

In the present study, hyperleukocytosis (defined as TLC >100.0×10⁹/L) was found in 8 (7.8%) patients. Studies from other Indian centers reported hyperleukocytosis in 15.3–23.2% cases [6,10]. Silverman et al. reported hyperleukocytosis in 10.8% of the ALL cases [7]. In our experience, the incidence of hyperleukocytosis was lower than the figures reported in other Indian centers and was similar to the prevalence reported from other nations. Children with TLC >50,000 were considered in the high-risk group.

Cytogenetic analysis
Mrozek et al. have described that the standard cytogenetic analysis can be obtained in most of the patients with ALL [11]. In large studies of adult ALL, between 70% and 75% of samples analyzed cytogenetically were deemed successful. Higher success rates, 83 and 91%, were reported by two large studies of childhood ALL [12,13]. Among successfully analyzed patients, one or more clonal aberration has been detected in 57–82% of children with ALL [12,13].

In our study, out of the 144 patients, successful karyotyping results were available in 131 (91%) patients and poor morphology in the remaining 13 (9%) patients. Waghray et al. in their study reported successful karyotyping in 52% of the cases [14]. Similarly, in a study by Yang, karyotyping was possible in 86% of the patients [15]. In another study by Forestier et al., cytogenetic analyses were carried out in 1372 (66%) patients. Among these, 787 (57%) displayed clonal chromosomal abnormalities [16]. Perez-Vera et al. in a study including 150 Mexican children aged from 5 months to 16 years with ALL reported successful karyotyping in 131 (87%) children [17]. These studies show that the successful karyotyping rate in our study was either comparable or better than most of the other studies. This could be due to stringent quality control and aseptic measures followed in our laboratory. Besides, most of the bone marrow samples received in the lab are from within the hospital and are transported in ambient temperature to the laboratory without any delay in time.

Whitlock and Gaynon have already explained in their study that both chromosome number (ploidy) and structural alterations have independent prognostic significance in childhood ALL [18].
have reported that 31% of the patients had a normal karyotype with hyperdiploidy (>47–50 chromosomes) in 17.5% of their children and 3.4% of their adults [21]. Pui et al., in 2008, have established that the rate of hyperdiploidy (2n>50) was associated with the most favorable prognosis compared to other cytogenetic groups [10]. Arico et al. have reported hyperdiploidy of more than 50 chromosomes in 17.5% of their children and 3.4% of their adults [21]. Pui et al., in 2008, have described that among all the chromosomal abnormalities identified in childhood ALL, hyperdiploidy (2n>50) was associated with the most favorable prognosis compared to other cytogenetic groups [10]. Arico et al. have established that the high hyperdiploidy (2n=51–65) generally occurs in cases with clinically favorable prognostic factors (patients aged 1–9 years with a low WBC count) and is itself an independent favorable prognostic factor [22].

A very useful explanation about the mechanism of gain or loss of chromosome has been described by Pederson-Biergaard and Rowley in 1994 [23]. They described that non-disjunction at mitosis may be the development of a haploid karyotype with a very high number of normal karyotype results, i.e., approximately 70% of patients.

The rate of hyperdiploidy in our study was 12.5%, which included both hyperdiploidy with and without additional change. Our findings were comparable to the study done by Amare et al. who had a hyperdiploidy rate of almost 15.4% [20]. In fact, in their study, they found a lower frequency of hyperdiploidy (15.4%) and a higher frequency of hypodiploidy (38.4%). Similarly, Li et al. have reported hyperdiploidy of more than 50 chromosomes in 17.5% of their children and 3.4% of their adults [21]. Pui et al., in 2008, have described that among all the chromosomal abnormalities identified in childhood ALL, hyperdiploidy (2n>50) was associated with the most favorable prognosis compared to other cytogenetic groups [10]. Arico et al. have established that the high hyperdiploidy (2n=51–65) generally occurs in cases with clinically favorable prognostic factors (patients aged 1–9 years with a low WBC count) and is itself an independent favorable prognostic factor [22].

In the present study, normal karyotype was observed in 91 (69.46%) patients; pseudodiploid in 15 (11.45%) patients; hyperdiploidy (~47–92 chromosome) in 15 (11.45%) patients, hypodiploid with additional change in 3 (2.29%) patients, hypodiploid (~46 chromosome) in 4 (3.05%) patients, and hypodiploid with additional change in 3 (3.05%) patients. Our findings are inconsistent with previous studies. Waghray et al. have reported that 31% of the patients had a normal karyotype with hyperdiploid karyotype in 38% of the patients [14]. Gladstone et al. found hypodiploidy in 63.3%, pseudodiploidy in 20%, and hypodiploidy in 6% of patients [19]. In contrast to these studies, our study had a very high number of normal karyotype results, i.e., approximately 70% of patients.

Table 4: Comparison of karyotype and FISH analysis

| S. No. | Clinical diagnosis | Karyotype | FISH |
|--------|-------------------|-----------|------|
|        |                   |           | TEL/AML | MLL | BCR/ABL |
| 1      | Pre B-ALL         | 46, XX    |       |     |         |
| 2      | Pre B-ALL         | 45, XY, der (9;12) |       |     |         |
| 3      | Pre B-ALL         | 46, XY, t(9;22) (q34;q11) | +     |     |         |
| 4      | Pre B-ALL         | 46, XY, t(10;14) (q24;q11) | –     |     | +       |
| 5      | Pre B-ALL         | 46, XY, der(1), del(7p)[15]/46, XY[2] | –     |     |         |
| 6      | Pre B-ALL         | 46, XX, der (9;22) (p13;q22) | +     |     |         |
| 7      | Pre B-ALL         | 46, XX    |       |     |         |
| 8      | Pre B-ALL         | 46, XY, t(9;22) (q34;q11) | –     |     | +       |
| 9      | Pre B-ALL         | 46, XY    |       |     |         |
| 10     | Pre B-ALL         | 46, XY    |       |     |         |
| 11     | Pre B-ALL         | 46, XY, 11q23 | –     |     |         |
| 12     | Pre B-ALL         | 46, XY, t(9;22) (q34;q11), del (9p) | –     |     | +       |
| 13     | Pre B-ALL         | 46, XY, (t12;21) (p13;q22) | +     |     |         |
| 14     | Pre B-ALL         | 46, XX, (t12;21) (p13;q22) | +     |     |         |
| 15     | Pre B-ALL         | 46, XY    |       |     |         |
| 16     | Pre B-ALL         | 44, XY, 11q23 | –     |     |         |
| 17     | Pre B-ALL         | 47, XY, (t12;21) (p13;q22) | +     |     |         |
| 18     | Pre B-ALL         | 46, XY    |       |     |         |
| 19     | Pre B-ALL         | 45, XX, t(9;22) (q34;q11) | –     |     | +       |
| 20     | Pre B-ALL         | 46, XY    |       |     |         |
| 21     | Pre B-ALL         | 46, XX, t(12;21) (p13;q22) | –     |     |         |
| 22     | Pre B-ALL         | 46, XY    |       |     |         |
| 23     | Pre B-ALL         | 46, XY    |       |     |         |
| 24     | Pre B-ALL         | 46, XY, t(12;21) (p13;q22) | +     |     |         |
| 25     | Pre B-ALL         | 46, XX, t(12;21) (p13;q22) | +     |     |         |
| 26     | Pre B-ALL         | 46, XX, t(12;21) (p13;q22) | +     |     |         |
| 27     | Pre B-ALL         | 47, XX, +mar[3]/46, XX, (t12;21) (p13;q22) | +     |     |         |
| 28     | Pre B-ALL         | 46, XY, t(12;21) (p13;q22) | +     |     |         |
| 29     | Pre B-ALL         | 46, XY, t(9;22) (q34;q11) | –     |     | +       |
| 30     | Pre B-ALL         | 46, XY    |       |     |         |

Table 5: Summary of the FISH results for ETV6/RUNX1, BCR-ABL, and MLL gene and its comparison with karyotyping result

| Chromosome abnormalities | Total number of patients (%) |
|-------------------------|------------------------------|
|                         | Negative by karyotyping and FISH | Positive by karyotyping and FISH | Positive by FISH only | Variant | Hyperdiploidy | Hypodiploidy |
| t(12;21) (ETV6/RUNX1) (n=30) | 17 (56.66) | 13 (43.3) | 4 (13.33) | 3 (10) | 8 (26.66) | ETV6-6 (7%) |
| t(9;22) BCR/ABL1 fusion (n=30) | 25 (83.33) | 5 (16.66) | 1 (20) | 1 (3.33) | |
| Break-Apart MLL gene 11q23 (n=30) | 26 (86.66) | 4 (13.3) | 2 (50) | 0 | 1 (3.33) | 1 (3.33) |
gain of certain chromosomes or multiple losses by non-disjunction. In addition to non-disjunction, chromosome lagging, formation of micronuclei, deletion of parts of chromosomes or telomeric loss may also result in chromosome loss or gain of the chromosome. Onodera et al. have described that the hyperdiploid karyotype, which arises either by doubling of chromosomes from a near-haploid karyotype or gain of chromosomes from a diploid karyotype during a single abnormal cell division [24].

We reported 7 (4.9%) cases of hypodiploidy with or without any additional changes. Our findings corroborate with literature which says that the hypodiploidy is observed in 3–10% of adults and 1–7% of childhood ALL [25]. Li et al. in their study found that 4.3% of children and 4% of adults showed hypodiploidy with 40–45 chromosomes [21]. Pui et al. have described that a progressively worse outcome is associated with a decreasing chromosome number (hypodiploidy) [10]. Chessek et al. showed that patients with near haploidy (1%), hypodiploidy (9%), and low hypodiploidy (1.6%) had a relatively poor prognosis [12].

We observed thirty-five (26.7%) structural abnormalities in our patients that included 9 (25.7%) translocations, 2 (5.7%) inversions, 11 (31.4%) deletions, 2 (5.7%) isochromosome, 1 (2.85%) duplication, 2 (5.71%) additions, and 8 (22.8%) derivative chromosomes. Pui et al. have described that nearly half of childhood ALL have chromosomal abnormalities in the form of translocations, which are nearly equally divided between random and nonrandom rearrangements [26]. Among the translocations, we observed that the most common translocation was t(9;22) in 5 (14.28%) patients. Padi et al. in their study carried out on 31 subjects observed that translocations as their major structural abnormality [27]. The t(9;22) was observed in approximately 10% of their patients.

Fish analysis

FISH can be easily performed on specimens prepared for cytogenetic studies, is more sensitive, able to diagnose cryptic rearrangements, and provides rapid results. Application of FISH, therefore, represents an efficient and effective strategy to maximize the information obtained from clinical specimens. In our setting, as a routine, we usually perform karyotyping on all ALL samples and FISH for ETV6-RUNX1 fusion, BCR-ABL and MLL rearrangements for pre B-ALL patients.

The t(12;21) (p13;q22) involving the RUNX1 (AML1 and CBFA2) gene is the most frequent translocation in children with ALL. This translocation is present in 25% of childhood precursor-BALL and 2% of adult precursor-B-ALL and is correlated with a moderate to favorable prognosis [28-30]. Although this translocation can be detected by both conventional karyotype and FISH, according to "Acute Lymphoblastic Leukemia Best Practice Guidelines (2011)" V1.006, it is mandatory to perform FISH testing for ETV6/RUNX1 in all infants and pediatric cases due to its cryptic nature and its prognostic significance. ETV6/RUNX1 probe, in addition to detecting cryptic t(12;21) (p13;q22) translocation also detects: (i) Amplification of RUNX1 which signifies intrachromosomal amplification of chromosome 21 (IAMP21) and (ii) extra signals of RUNX1 which suggests hypodiploid karyotype.

Interphase FISH testing for confirmation of high hypodiploidy should be performed by including probes for chromosomes X, 4, 6, 10, 14, 17, and 18 especially in cases when a normal karyotype is obtained, and interphase FISH identifies extra signals for RUNX1 or where chromosome analysis is unsuccessful. The t(12;21) (p13;q22) (cryptic on karyotyping) in the production of a fusion protein that acts in a dominant-negative pattern and inhibits the transcription of RUNX1 gene. After the first detection of the ETV6-RUNX1 fusion by FISH, a large number of studies demonstrated that the t(12;21) is rarely the only abnormality present. Additional abnormalities include del(6q), Del(11q), rearrangements of 12p, and del(16q), and often these abnormalities provide a clue that a t(12;21) might be present. The t(12;21) has a favorable prognosis with cure span ≥90%, especially if other favorable factors are present. The frequency of this translocation has been found in a range from 14% to 25% by molecular techniques [31]. In our study, we detected t(12;21) (p13;q22) with a frequency of 43.3% which included detection by either of the techniques. Therefore, we reported a higher incidence of ETV6/ RUNX1 involving B lineage ALL than previously reported studies in this region [32]. It is worth noting that the lower frequency of this fusion gene has also been observed in other studies from within India (6%), Mexico (9.6%), Argentina (11.6%), Thailand (12%), China (17.9%) and Taiwan (19%) [33-38]. The higher frequency of ETV6/RUNX1 gene along with hypodiploidy provides a clue to the higher frequency of molecular subgroup of leukemia with a potential for favorable clinical outcome in precursor B-ALL from the North India. This is in complete contrast with the study done by Inamdar et al. from Bombay, and Padhi et al. from Southern India who found a very low frequency of B-ALL with favorable clinical outcome [27,33]. These geographical variations within India can be explained on the basis that the prevalence of this gene in the North Indian population per se could be more as compared to other parts of India. Furthermore, India is a big country with diverse social and cultural population, thereby bringing heterogeneity in the patient population as well. The difference in the patients' inclusion criteria could also be a reason for this high incidence.

For 30 patients, it was possible to perform both karyotype and FISH panel for BCR-ABL gene in our study. The Philadelphia (Pb) chromosome is derived from the t(9;22) (q34;q11.2). The incidence of t(9;22) in childhood ALL varies from 3% to 5%, but various authors have reported its incidence ranging from 2% to 50% [39-43]. At the molecular level, the breakpoints in B-ALL and CML differ, and this variation leads to the production of p190 and p210 fusion proteins, respectively. In our study, both karyotype and FISH analysis were positive for BCR-ABL gene in 5 (16.66%) out of 30 patients which is in concordance with other studies [39-43].

Russo et al. found partial or complete monosomy seven in approximately 25% of 57 children with Ph+ ALL [44]. In our study, although the positivity rate of this fusion was low (5 patients, 16.66%), we did observe monosomy seven in two out of 5 patients (40%). This observation is notable because the loss of one copy of chromosome seven generally characterizes myeloproliferative disorders that progress to acute myeloid leukemia. This subgroup of children with Ph+/−7 ALL comprised mainly older males with early B-lineage ALL, whose induction failure rate (31.6%) was much higher than that than other Ph-cases. These findings suggest that leukemia transformation in such patients is a multistep process involving the interaction of a dominant oncogene (Ph; BCR-ABL) with a tumor suppressor gene (−7). In both children and adults, t(9;22) ALL has the worst prognosis among patients with ALL. Oyekunle et al. have described that the deletions of the IKZF1 gene confer an adverse risk profile in Ph-positive ALL [45]. The IKZF1 gene has a coding function for a transcription regulator involved in T- and B-cell differentiation.

In our study, it was not possible to perform both karyotype and FISH panel for MLL gene in 30 patients due to some reasons. The detection of MLL rearrangements by karyotyping is although sensitive but sometimes problematic, especially, when the MLL-rearrangement is a subtle anomaly, and chromosome preparations are of poor quality. The duplications and deletion involving the MLL genes are even more difficult to detect by karyotyping. For translocations detection, irrespective of the translocation partner, FISH is the method of choice [46]. In our study, out of the four patients positive for MLL rearrangements, two patients (50%) were detected only by FISH analysis. We, therefore, highly recommend the use of FISH for the detection of MLL rearrangements to overcome the shortcomings associated with the karyotyping.

We found that the frequency of MLL rearrangements was up to 13.3%. This was quite high as compared to study carried out by Safaie et al. attributed their low frequency of 1.5% to the fact that the occurrence of this abnormality is more frequently seen in infants under 1 year.

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and secondary ALL [47]. Because our positive sample size was too low, we cannot say for sure whether this was a chance occurrence or not. **MLL** translocations in ALL are associated with the pre-B-ALL immunophenotype (CD19+/CD10-) and are characterized by a poor prognosis, particularly in infants [48].

**Survival analysis**
The estimated OS for the entire series at 5 years was 75%. Advani et al. documented OS, EFS, and DFS at 5 years of 53%, 60%, and 49%, respectively, in their study from TMH between 1986 and 1993 [6]. Silverman et al. in a study found that OS and EFS of 88±2% and 83±2%, respectively, with no difference in OS according to standard and high-risk groups [7]. In another study, it was reported that 6 years EFS in cases of M2 or M3 bone marrow at day 33 of induction was only 11±5% [49]. Trigg et al. from Children Cancer Study group documented a 10 years OS and EFS of 73% and 62%, respectively, between 1985 and 1989 [50]. Horibe et al. from Japan documented 7 years OS and EFS rates of 76±1.9% and 61.4±2.1%, respectively [51]. Shing et al. from Hong Kong documented 7 years OS of 67–80% depending on the presenting leukocyte count and that the results were comparable to the MRC UKALL X trials [52]. Thus, results from our hospital are significantly comparable to other series from affluent countries.

Leukemia is a disease with heterogeneous causes and with well-defined cytogenetic molecular abnormalities inducing clinical manifestations [53]. Leukemic cells have a very challenging heterogeneous environment with different receptivity to prescribed drug or chemotherapeutic agents [54]. Therefore, accurate diagnosis using molecular cytogenetics and other advanced techniques plays a crucial role in the overall survival of the patient.

This study has some limitations. First, not all children were tested with all diagnostic techniques and therefore, the sample size for comparison between the two techniques, i.e., karyotyping and FISH was quite low. Further studies with increased sample size would be worthwhile to analyze the two techniques. Second, advanced molecular methods to detect subtle abnormalities, including chromosomal microarray, were not available to substantiate our observations. Finally, many patients were lost to follow-up, and therefore, a complete picture of the disease course cannot be said with confidence.

**CONCLUSION**
We found that normal karyotypes in our study population were more frequent. Our study indicated that employing FISH technique with increased sensitivity helps in detecting various chromosomal abnormalities, more so with the cryptic rearrangements. Observation of monosomy seven in two out of **BCR-ABL** positive 5 patients (40%) is notable, because the loss of one copy of chromosome seven generally characterizes myeloproliferative disorders that progress to acute myeloid leukemia and suggest that leukemic transformation in such patients is a multistep process involving the interaction of a dominant oncogene (Ph: **BCR-ABL**) with a tumor suppressor gene (-7). Unlike other studies both in India and in other countries, higher frequency of molecular subgroup of leukemia with a potential for the favorable clinical outcome (**ETV6-RUNXI**, hyperdiploidy) in precursor B-ALL was observed from the North India. With the availability of NGS and other techniques, we understand the human genome variability and its impact on disease susceptibility and drug response. With the availability of multiple genomic panels for investigation, the whole scenario of diagnosis and decision-making is changing. However, cytogenetics analysis including karyotyping and FISH with clinical details are gold standard, and we recommend their use to provide a more accurate and reliable characterization of ALL for better prognosis and best possible clinical outcome with improved cure rates and decreased drug toxicity.

**ACKNOWLEDGMENT**
We greatly acknowledge all the patients who kindly consented to be a part of the study. The authors also thank the whole-hearted support and encouragement from the management of Indraprastha Apollo Hospitals, and Dr. Ashok K Chauhan, Founder President, Amity Group of Institutions.

**AUTHORS’ CONTRIBUTIONS**
Dr. Mohit Chowdhry, Dr. RN Malikoo, and Dr. Pankaj Sharma participated in the conception, design, and coordination of the study; Manoj Kumar, Deepika Rani, and Vandana Sharma performed the study; Manoj Kumar, and Dr. Mohit Chowdhry drafted the manuscript.

**CONFLICTS OF INTEREST**
The authors report no conflicts of interest.

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