Discussion
In general, the prognosis of ALL in the developing world remains poor due to a multitude of adverse clinical and social factors, the most prominent among these being the lack of resources available to both patients and health care professionals [Magrath IT. et al; 1997]. This often leads to delay in diagnosis, which may influence tumor burden and treatment outcome. The impact of delayed diagnosis on outcome (as well as on the clinical presentation) is, however, difficult to quantify. In addition, differences in the environment and lifestyle as well as potential genetic differences may influence the incidence and outcome of ALL. Thus, in order to improve the survival of patients with ALL in developing countries, it is important to conduct research into the biology, response to treatment and prognostic factors in the developing countries themselves. In the present series, patients with ALL in a single Indian institution were studied for cytogenetics analysis and treated uniformly with standard protocols in order to identify problems and prognostic factors that might be specific to this patient's population.

Scope of cytogenetic studies in Leukemia

Since its first application to the study of cancer, cytogenetics has taken us from a state of virtually no knowledge of the chromosome changes in human cancer to a point at which a staggering body of information is available. The latter is evidenced by increasing number of aberrations and identification of more than 1078 fusion genes in neoplasia [Mitelman F. et al; 2013]. Therefore, more than half a century old, the field of cancer cytogenetics has more than lived up to its envisioned task of finding recurrent or specific abnormalities associated with cancer and continues to provide crucial diagnostic and prognostic information. In current practice, cytogenetic data often serve as a guide in other studies, ranging from the exploration of conventional cytogenetic findings with various methodologies, singly or in combination with molecular cytogenetics [Sangbarg A. et al; 1999, Seyed Hashem MM. et al 2012].

There is continuing interest in understanding what causes identical translocations to yield different effects, a phenomenon seen particularly in ALL [Swerdlow SH. et al; 2008]. In the present study, detection of abnormalities in leukemia was useful not only for diagnostic and treatment purposes but also for prognostic risk assessment.
The cytogenetic data of the present study may also provide key background information for the recognition and identification of genes (and their networks) involved in cancer and for their subsequent application in therapeutic development.

**Scope of cytogenetics in ALL**

ALL harbor a variety of recurrent genetic aberrations, some of which can be ascertained by cytogenetic analysis [Haferlach T. et al; 2005, Mrozek K. et al; 2004, Meyer MS. et al; 2006]. A subset of cytogenetic abnormality in ALL is diagnostic of specific entities and has independent prognostic value, which directly impacts therapeutic decision making [Swerdlow SH. et al; 2008]. Treatment decision making (including remission induction chemotherapy) depends on multiple parameters; however, genetic data are paramount.

Cytogenetic abnormality is an independent prognostic factor for duration of OS and DFS even when age, initial leukocytic count (WBC), FAB sub type, and immunologic phenotype are considered [Bloomfield CD. et al; 1986]. Therefore, the detection of chromosome abnormalities by CC is an important component in assessing the classification and risk stratification of ALL [Moorman AV. et al; 2007, Pullarkat V. et al; 2008, Seyed Hashem MM. et al; 2012]. Although many of the translocations can be detected (and indeed were discovered) by CC, now a days molecular cytogenetic especially FISH for detection of specific abnormalities are becoming routine in the clinics [AMP test directory 2012].

The first major study demonstrating the independent prognostic significance of cytogenetic findings at diagnosis in ALL was the Third International Workshop on Chromosomes in Leukemia [Third international workshop; 1981, Bloomfield CD. et al; 1986, Bloomfield CD. et al; 1989]. Subsequent studies confirmed the workshop’s results and refined them by providing data on clinical relevance of further recurrent aberrations and elucidating the molecular basis and biologic consequences of many of these aberrations.

Earlier studies on ALL cytogenetics mainly consisted of smaller or larger series but specifically on aspects viz. children or adults or older age above 60 year or only selective cytogenetics categories mostly from developed nations [Pullarkat et al;

The present work gives the first large series of unselected ALL, providing an idea about the distribution of ALL subtype in West Indian population. Almost patients with ALL in Gujarat, Rajasthan and few region of Uttar Pradesh are treated at Gujarat Cancer and Research Institute, Ahmedabad. Current practice is to stratify patients according to a very small number of prognostic variables that are exclusively established as having an important influence on outcome.

**Etiology and Epidemiology:**

In the present study, total 273 ALL patients were characterized by cytogenetic analysis to identify their role in risk stratification and significances of particular abnormality. There were 193 (70.7%) males and 80 (29.3%) females. In the present study, the patients presented a wide age range from 4 months to 75 years (Figure 81) and mean age was 17.7 years.

![Age distribution of ALL patients](image)

*Figure 81: Age distribution of ALL patients in the present study.*

Male to female ratio in the present study was 2.4:1 which showed slight male predominance, which is slightly higher in accordance to previous reports by Venkateswaran SP. et al (2012) (1.4:1) at Kerala, India and quite similar reported by other centers in India and elsewhere [Geeta V. et al; 2012]. That is may be due to that present study not follows any selection bias criteria. However, Venkateswaran
SP. et al. presented study based on selected patients by Immunophenotype report and cytogenetics study.

The reported median age range of ALL patients is 14 years worldwide [SEER, NCI 2012]. In present study median age observed was 13.5 years. Worldwide reported that ALL most common in childhood with a peak incidence at 2-5 years of age [SEER, NCI 2012, Figure 4]. In present study, the higher incidence of ALL was observed in age range 1-5 years (59 patients) (Figure 81) which is quite similar with SEER report. ALL in infants is rare and constitutes only approximately 2-3% of all ALL patients [SEER, NCI 2011]. In present study there were 3% infant patients observed out of 273 patients. In present study there were 142 (52%) children observed out of 273 patients.

Any precise etiological factor for ALL with any of the patients was not observed in the present study.

Assessment of cytogenetic categories in present study

The patients were categorized into six main cytogenetic categories (Table 12). The first category comprised 27 Philadelphia positive patients. Philadelphia positive patients were categorized separately with respect to cytogenetic categories and their survival because the presence of the Philadelphia chromosome was used to direct therapy. This approach meant that the prognostic relevance of cytogenetic categories could be ascertained accurately without their effect being masked by the strong and established poor outcome associated with Philadelphia positive ALL [Moorman AV. et al; 2007]. Shaikh MU. et al (2011) have also reported 15% Philadelphia positive patients as separate category.

The second category comprised 10 patients of hyperdiploidy with t(9;22). The simultaneous presence of additional karyotypic abnormalities like Philadelphia positive and hyperdiploidy may alter the biological properties of cells and influence clinical outcomes [Tauro S. et al; 2003]. Yanan Li. et al (2009) also reported 13 patients of hyperdiploidy with t(9;22) showed inferior outcome in their study. Therefore in the present study such 10 patients were considered as an individual category.
In the present study, The patients having hyperdiploidy (2n+) with t(9;22) (median age 37 years) were older than the others categories (Table 12, Figure 31) and the estimate OS was (1.40%) lowest then other cytogenetic categories (Table 13, Figure 40), thus age reflecting the survival disadvantage. Present study showed that age is probably the most important prognostic factor. This is in accordance to Gökbüget N. et al (2006) observation.

According to Charrin et al. (2004) patients with hypodiploidy and hyperdiploidy represent 2 sides of a distinct biological subgroup. Charrin et al. (2004) reported that patients with Hypodiploidy showed inferior outcome. Moorman AV. et al. (2007) also reported significantly reduced OS in hypodiploid patients as compared to hyperdiploid patients. Therefore, in the present study hyperdiploid and hypodiploid patients separated into two categories. The third category comprised of 26 patients with hyperdiploidy and fourth category comprised of 20 patients with hypodiploidy.

The fifth category comprised of 149 (54.6%) patients with normal karyotype. According to Lafage M. et al (2003), the presence of normal metaphases could be explained as residual normal cells, the marrow infiltration by leukemic blasts being usually partial; it could also be the result of the low mitotic rate of the blast cells.

Result of present study was in contrast to that reported by with Perez et al (2001) who reported only 30% of their cases with normal karyotype. However this was in accordance with that reported by Mesquita DR. et al (2009) who reported 56.82% cases with normal karyotype in children with B-lineage lymphoblastic leukemia in Brazil’s federal district. Venkateshwaran SP. et al (2012) reported normal karyotype in 70% patient in kerala, India.

In present study the proportion of normal karyotype (54.6%) were decrease in non-selected Indian ALL patients as compare to Brazil’s children and kerala’s patients, likely attributable to technical progress such as improvement of culture conditions and the introduction of FISH.

The sixth category comprised of 41 patients classified as “miscellaneous category”, because some chromosomal abnormalities were observed but were too infrequent.
to be analyzed separately (Table 11). Similar observations have been also reported by Anthony V. et al (2009) in patients with miscellaneous category.

**Cytogenetic risk group assessment**

In the present study, all 273 patients were divided into 3 cytogenetic risk groups for chromosomal analysis according to WHO classification. Majority of patients were observed in favorable risk group (186 patients) followed by adverse risk group (79 patients) and intermediate risk group (8 patients) (Table 13). Similar risk group have been also presented by Pullarkat V. et al (2008) in ALL patients in previous study.

Pullarkat V. et al (2008) reported older age and higher WBC counts in adverse group patients among studied patients. In the present study age was observed higher for those with adverse group patients (mean age 24.34 years) among the 3 risk groups (p<0.0001) (Figure 33). However, the WBC counts were higher in intermediate risk group (mean WBC count 107x10³/cmm) among the all risk groups (Figure 35) (p<0.001). This could be due to the small number of patients we have compared with the huge diversity of cytogenetic abnormalities described in the literature so far.

**Assessment of various cytogenetic abnormalities**

**Numerical chromosomal abnormalities**

In the present study, incidences of hypodiploidy were most probably equal among children and adult patients. The major differences were observed for the other categories; high hyperdiploidy (15%, 7/46) and hyperdiploidy (24%, 11/46) were much more frequent among patients 1 to 15 years of age (Table 14).

**Hyperdiploidy**

In the present study results based on ploidy are similar to those reported in the literature (Table 14, Figure 82).
Discussion

Literature (\%)  

Present study (\%)

Figure 82: Comparison of ploidy status in Literature and present study.

In literature high hyperdiploidy was reported 25-30\% of the ALL children [Mrozek K. et al; 2004, Paulsson K. et al; 2009, Harrison CJ. et al; 2002], whereas in present study 23.3\% were observed. In literature high hyperdiploidy was reported only in 10\% of adult patients [Mrozek K. et al; 2004, Moorman AV. et al; 2007, Faderl S. et al; 1998], whereas in present study 12.5\% adults patients were observed with high hyperdiploidy. In literature hyperdiploidy were reported in ~30\% [Mrozek K. et al; 2004, Kwon YO. et al; 2009] of childhood ALL and in 15-25\% [Mrozek K. et al; 2004, Seeker-walker LM. et al; 1997] of adult patients. In present study hyperdiploidy were reported in 36.7\% of childhood and 31.3\% of adult patients. In literature near-triploidy is rarely reported (less than 1\%) in childhood ALL [Mrozek K. et al; 2004, Pui CH 1990] and represented only 3-5\% of the adult cases [Seeker-walker et al;1997, Kwon YO. et al; 2009]. In present study near-triploidy were reported in 1\% of childhood and 3\% of adult patients. The most common chromosomes gained in hyperdiploidy (chromosomes 4, 5, 6, 8, 11, 12 and 21) belonged to the same pairs as reported in the literature [Forestier E. et al; 2000, Kwon YO. et al; 2009].

The most common trisomies in the present study

Trisomy 8: The trisomy 8 is likely to be a disease-modulating secondary event, with underlying cryptic translocations, deletions, or mutations as primary events. Genes
with possible significance in leukomogenesis located on chromosome 8 include \textit{C-MYC} on 8q24, \textit{C-MOS} on 8q22, \textit{MOZ} on 8p11, and \textit{ETO} on 8q22. Trisomy 8 could represent an alternative mechanism for increasing \textit{C-MYC} gene dosage to achieve amplification of \textit{C-MYC} oncogene [Bakshi SR et al; 2012]. Trisomy 8 is more often found in B-cell than in T-cell cases. Trisomy 8 is a rare anomaly in lymphoid malignancies (90% of trisomy 8 occur in myeloid malignancies); found in about 5% of ALL. Rarely found as a sole anomaly (5-10%), may be part of hyperdiploid karyotypes (>50 chromosomes mainly) without structural anomalies (20% of cases), mostly found in complex karyotypes with structural anomalies (2/3 of cases), these complex karyotypes being often hyperdiploid as well sex ratio: 1.5/1. accompany (mostly in complex karyotypes): t(9;22)(q34;q11), t(4;11)(q21;q23) and other 11q23, del(6q), t(1;19)(q23;p13), dic(9;12)(p13;p12) and other known primary anomalies. [Huret JL. 2007].

In the present study most frequent numerical change i.e. gain of chromosome 8 was observed in 9 (3.3%) patients. Among that, 7 were male and 2 were female patients. Among that, 3 patients from t(9;22)(q34;q11.2) with hyperdiploidy category showed other structural anomalies, one with i(17)(q10), another two with del(20)(q) and +der(20) respectively (Table 16, Case no. 29, 30, 35). One patient observed in miscellaneous category with add(13)(p11) (Table 11, Case no.15). Five patients observed in hyperdiploidy category (Table 15, Case no.2, 3, 4, 8, 9, Figure 38).

Prognostic significance of trisomy 8 in lymphoid malignancies is not reported widely. Thus, the presence of trisomy 8 in ALL poses a question for its role in leukomogenesis. However in the present study, sole trisomy 8 was observed in only patient no. 2 and other 4 patients were observed with additional numerical abnormalities. Total 6 patients were persistent with disease and expired during treatment. Two patients were relapsed and expired. That is may be due to presence of additional structural and numerical cytogenetic changes lead to effect in prognosis. Only one patient with sole trisomy 8 was alive and relapse after 41 month survival. Therefore prognostic significance of trisomy 8 from present study is inconclusive.
**Trisomy 5**: In literature trisomy 5 is reported in both ALL and AML. Trisomy 5 as a sole abnormality in ALL is very rare and described in only 17 cases, including 4 female patients and 13 male patients [Mitelman; 2013]. In childhood ALL, an extra chromosome 5 is commonly encountered in cases with hyperdiploidy >50 chromosomes [Ma ESK. et al; 2002]. In the present study gain of chromosome 5 was observed in 3 patients (Table 15, Case no. 5, 8 and 10, Figure 41). It was observed in one adult female patient as secondary abnormality in CCR and in 2 male children with sole abnormality.

The presence of trisomy 5 in high hyperdiploid childhood ALL is associated with a less favorable clinical outcome. Trisomy 5 in childhood ALL with hyperdiploidy >50 chromosomes is associated with a poorer clinical outcome [Ma ESK. et al; 2002]. In the present study, among 3 observed patients, female adult patient with hyperdiploidy (> 50 chromosomes) with complex chromosomal abnormality was persistent to chemotherapy and 2 male children were expired during treatment therefore it was associated with a poorer clinical outcome.

**Trisomy 21**: Trisomy 21 is the frequent aneuploidy observed in both adult and childhood ALL. Its overall incidence would be around 15% of cases. As the sole clonal abnormality, trisomy 21 accounts for 2% of pediatric and less than 1% of adult ALL cases. In childhood ALL, the incidence of trisomy 21 is approximately of 40% and of 80%, respectively, in the 47-50 chromosomes and in the >50 chromosomes ploidy groups. The main association is with t(12;21)(p13;q22) in childhood (15% of cases at diagnosis), followed by 6q abnormalities. It is also associated with t(1;19)(q23;p13), t(4;11)(q21;q23) and 14q abnormalities. Essentially observed in B-cell lineage [Viguié F. 2001]. In present study gain of chromosome 21 was observed in 3 patients among that there were one male and two female (Table 15, Case no. 1, 4 and 8, Figure 37). Two children and one adult were observed, one female patient observed with high hyperdiploidy and there was no structural rearrangement observed in any patient with trisomy 21.

In the group 47-50 chromosomes, trisomy 21 has a rather good prognosis in children, when it is not associated with a bad prognosis structural rearrangement. In the same
ploidy group, trisomy 21 has no prognostic impact in adults [Vigué F. 2001]. However, in the present study all 3 patients were persistent to disease during chemotherapy therefore it was associated with a poorer clinical outcome.

**Trisomy 6**: Results of present study showed gain of chromosome 6 in 2 patients. Trisomy 6 was reported in both male patients in addition of trisomy 8 chromosome (Table 15, Case no. 3 and 4). Among these 2 patients one was 55 year old and another was 2 year old. Both patients were persistent to therapy.

**Trisomy 12**: in the present study trisomy 12 was observed in male pediatric and female adult patient (Table 15, Case no. 6 and 8). In Female patient in addition to trisomy 12, trisomy 5, 8, 16 and 21 were observed. Female patient was persistent to disease during chemotherapy and male patient was lost to follow up during treatment.

However, in the literature rare instances of trisomy 6 and 12 encountered in childhood acute mixed lineage leukemia, lymphoblastic transformation of CML, and chronic myeloproliferative disorder [Ma ESK. et al; 2004].

**Trisomy 4**: In literature, trisomy 4 has been described in five cases of ALL as the sole chromosomal anomaly [Mitelman; 2013] and combined trisomies of chromosomes 4 and 10 are found in children with B-progenitor cell ALL with a favorable prognostic association [Beghini A. 2000]. In the present study, trisomy 4 observed in addition to gain of chromosome 7, 8, 17 and 18 in 2 year male patient (Table 15, Case no. 9). Patient was relapse disease and expired at the end of 2\textsuperscript{nd} maintenance cycle of treatment protocol.

**Trisomy 18**: Trisomy 18 is common in hyperdiploid ALL with more than 50 chromosomes (15-27% of cases). The great majority of karyotypes with trisomy 18 also exhibit trisomy 4, 6, 10, and 14, either trisomy 21 or tetrasomy 21, and an extra X chromosome. More than half either have trisomy 17 or an isochromosome 17q [Heerema NA. et al; 2000]. In the present study, trisomy 18 observed in high hyperdiploid 2 year male patient in addition to gain of chromosome 4, 7, 8 and 17.
It is unusual to see trisomy 18 in a hyperdiploid ALL with fewer than 50 chromosomes. It is likewise unusual to find trisomy 18 associated with one of the common structural changes in ALL, such as the t(1;19). Among 13 reported ALL cases, with trisomy 18 as the sole cytogenetic abnormality [Mitelman; 2013], nine were reported from India. Is there an environmental component to this unusual distribution of cases?

The prognosis appears to be neutral to favorable in a karyotype with >51 chromosomes that includes trisomy 18. There is some evidence of an unfavorable prognosis if the karyotype is isolate trisomy 18 [Van Dyke DL. 2003]. In the present study patient observed with trisomy 18 was relapse disease and expired at the end of 2nd maintenance cycle of treatment protocol (Table 15, Case no. 9).

**Hypodiploidy**

The prevalence of hypodiploidy is roughly equal among childhood and adult cases, at 5-6% [Seeker-walker et al; 1997, Group francais; 1996, Heerema NA. etal; 1999]. Raimondi et al (2003) reported hypodiploidy is a very heterogeneous category and occurs in 6-7% of patients with childhood ALL.

In the present study, hypodiploid group comprised 20 patients (Table 12). Present study showed that hypodiploidy occurs infrequently in 7.3% (20/273) of all cases and 7.7% (11/142) of patients with childhood ALL. Patients with 45 chromosomes are the largest hypodiploid group. In present study, 10% hypodiploid (45 chromosomes) and 8.6% hypodiploid (<45 chromosomes) children among ploidy categories were observed (Table 14). Its prognostic significance has been controversial. In the initial period some investigators suggested that hypodiploidy at diagnosis is a favorable risk feature [Bloomfield CD. et al; 1981, Seeker-Walker LM. et al; 1982], while others have shown it to be associated with a poor treatment outcome [Nachman JB. et al; 2007, Coustan SE. et al; 2009, Raimondi et al; 2003, NCCN guidelines; 2012].

In the present study monosomy of chromosome 9 were observed in 2 children patients (Figure 43). They have additional monosomy 15 and 22, respectively. One was alive after 31 months survival and competed 2 maintenance cycle of treatment. Another patient was with persistent disease and expired after 4.1 months survival.
during therapy. Monosomy of chromosome 21 was observed in 2 patients. One was expired without report of relapse during treatment after 1.8 months. Another patient was with persistent disease and expired during treatment. In accordance to the literature [Raimondi et al; 2003], present study also indicates that monosomy associated with poor treatment outcome.

**Structural chromosomal abnormalities**

Total 78 patients with structural chromosomal abnormalities were observed during the present study (Figure 49).

The most common involved chromosome was 22, this was involved for 40 times. The second most common involved chromosome was 9, that was for 39 times. This is in accordance with Pui CH et al (1993), and Harrisson CJ. et al (2001), reported that abnormalities associated with chromosome 9 specially appear relatively frequently and are usually associated with poor prognosis and they represent one of the important risk factors.

Chromosome 19 anomalies could also be considered as a bad prognostic factor as about 2.6% of the present studied patients as compared to 6.5% in the literature [Raimondi SC. et al; 1995], having abnormal chromosome 19. Other chromosomes involved likewise, chromosome 1 for 12 times, chromosome 11 for 8 times, chromosome 6 for 7 times and chromosome 8 for 6 times. Chromosome 10, 14, 15 and chromosome Y was involved only for once. Chromosomes X was observed twice in studied patients. Involvement of chromosome 18 was not reported any time.

**Translocation (9;22):**

In the present study, the proportion of Philadelphia chromosome was detected in 37/273 total patients (Table 16). Among that, 3.5% (5/142) were observed in overall children and 26% (32/123) were observed in overall adults. This is quite similar to worldwide scenario [Schrappe M. et al; 2000, Roy A. et al; 2005, Ravandi F. et al; 2009, Litzow MR. 2009].

The detection of a Philadelphia chromosome remains a major prognostic factor of induction failure. Despite the steady improvement in the management of ALL in
Discussion

children, Philadelphia positive ALL is associated with high rates of relapse or resistance to treatment [Schultz K. et al; 2007, Oudot C. et al; 2008]. This disease is heterogeneous in terms of clinical parameters such as leukocyte count, age at diagnosis, and initial steroid response [Pulte D. et al; 2009, Arico M. et al; 2000]. A slow early response to conventional therapy has also been reported as indicative of a poor prognosis [Roy A. et al; 2005]. Philadelphia chromosome positivity, with an overall incidence of 20%–40% in adults, has an extremely poor prognosis [Gleibner B. et al; 2002, Masamitsu Y. et al; 2009]. Its incidence rises to 50% in patients aged ≥ 50 years [Larson RA. et al; 2006]. Present study also confirmed that a Philadelphia chromosome remains a major prognostic factor of induction failure observed in 49% (18/37) patients resistance to treatment and associated with high rates of relapse observed in 22% (8/37) patients.

Most CML present with Philadelphia chromosome as the sole cytogenetic abnormality and gains of secondary abnormalities are associated with accelerated phase or blast crisis and signifies progressive disease. However, the most Philadelphia positive ALL patients have secondary cytogenetic aberrations, which might influence the course of the disease and its response to treatment [Yanan Li. et al; 2009]. In the present study, additional chromosome changes were seen in 13(5%) patients (Case no 25 to 37, Table 16) among that, two patients had trisomy 8 (Case no 29 and 30, Table 16), one had trisomy 21 (Case no 33 and 33, Table 9), and one patient had i(17q) (Case no 29, Table 16). In 3 patients double Ph chromosome were observed (Case no 32 to 34, Table 16, Figure 45). Double Philadelphia chromosome is common in CML; it represents over expression of BCR-ABL fusion gene, but rarely found in ALL and associated with poor prognosis [Tauro S. et al; 2003]. In present study all 3 patients with double Philadelphia chromosome were male adult above 30 years age and with hyperdiploidy. All three were disease persistent and expired during first induction cycle of treatment course.

In the present study two patients were observed with mix clone population and one patient with variant signal pattern indicates deletion of 5’ABL gene (Case No. 35, 36 and 37, Figure 46 and 47). All three patients were resistant to treatment so re-induction cycle was introduced. Two patients were lost to follow up and one was
Discussion

expired before remission achieved. The inferior outcome of these patients may partially explain that one of the most common mechanisms of resistance to Imatinib is the mutation involving the ABL kinase domain and secondary aberration in Philadelphia positive patients [Yenan Li et al; 2009].

In the literature, hyperdiploidy (8-50%) were the most frequently reported additional cytogenetic aberrations in Philadelphia positive ALL patients [Heerema NA et al; 1999, Wetzler M et al; 1999]. In our series, only ten patients (4.5%) were of hyperdiploidy and it was much lower than the reports from western countries [Moorman AV et al; 2007, Yanan Li et al; 2009]. The only one study concerning the chromosomal abnormalities in Philadelphia positive ALL in Oriental people was from Taiwan reported in 18 patients (5.6%) with t(9;22) and hyperdiploidy [Ko BS et al; 2002] is close to our results.

1p36 rearrangement: In the present study, the second most common reported abnormality was; add(1)(p36) observed for 5 times (Case no 7, 8, 16, 17, and 19, Table 11, Figure 48A).

1p36 alterations are recurrent in hematological malignancies and present in various forms. The most frequent abnormalities in both myeloid and lymphoid neoplasias are unbalanced chromosomal rearrangements, usually described as add(1)(p36). The confirmed or putative tumor suppressor genes mapped to 1p36 include CDK11A (CDC2L2) and CDK11B (p58 or CDC2L1), TNFRSF1B (TNFR2), ID3, NBL1 (DAN), PAX7, TP73 and RUNX3. There are also oncogenes, including SKI and PRDM16. Other 1p36 genes, like MDS2, are involved in chromosomal translocations in hematological malignancies, but their role is still unknown [Duhoux FP et al; 2011]. In the present study, patients with 1p36 rearrangement, out of 5 patients, there were 2 male and 3 female, among that one 45 year old female was only disease persistent and other 4 children were in remission at last follow up.

Translocation (1;19)(q21;p13): In the present study t(1;19)(q21;p13) was observed in 3 patients (Case no 2, 18 and 20, Table 11, Figure 48G). t(1;19)(q21;p13)/TCF3(E2A)-PBX1 occurs in 1% to 3% of adult and 1% to 6% pediatric ALL, and can be in either balanced or unbalanced form, as der(19)t(1;19) with two
normal chromosomes 1. Almost all t(1;19)(q21;p13) patients are diagnosed with pre-B-cell ALL. Outcome of patients with t(1;19) is controversial [Mrozek K. et al; 2009].

Out of 3 patients, 2 were males (6 years and 26 years) and 1 female (2 years). Both males were diagnosed with B-cell ALL. Child was persistent to disease and adult was relapsed during treatment. Third patient was 2 years old female reach up to maintenance cycle during treatment and relapsed at last follow up, however all three patient with t(1;19) were expired during treatment. It shows that outcome was poor in patient with t(1;19) in present study.

11q23 rearrangement: In the present study 11q23 rearrangement was observed in 5 patients. t(4;11)(q21;q23) was observed in two patients (Case no 3 and 37, Table 11, Figure 48H). Translocations involving band 11q23/MLL are detected in two-thirds of infants with ALL. Their incidence in children and adults is much lower, 1% to 2% and 4% to 9%, respectively. The most common among 11q23 rearrangements is t(4;11)(q21;q23)/MLL-AFF1(AF4), detected in more than 50% of patients. The t(4;11) predicts a poor prognosis in children and adults, with particularly dismal outcome in patients with a poor early response to prednisone [Mrozek K. et al; 2009].

In the present study t(4;11)(q21;q23) observed in 25 years female and 16 years male patient. Female patient was relapsed and male patient was persistent to disease during treatment. In one male child patient (Case no 1, Table 18) CCR involving chromosomes 1, 4, 5, 11 were observed with 11q23 rearrangement. This abnormality has not been reported before. Patient was expired during treatment. Deletion (11)(q23) was observed in two patients (Case no 4 and 33, Table 11, Figure 48C), among that one patient was female and one was male. Both were children and expired during treatment. Therefore in present study all five patients with 11q23 abnormalities were associated with a poor clinical outcome and confirmed poor prognosis.

Deletion(6)(q21): Del(6q) occurs in 3% to 7% of adult and 6% to 9% pediatric ALL. The del(6)(q) predicts a intermediate prognosis in children and adults [Mrozek K. et al; 2009]. In the present study del(6)(q21) observed in two patients (Case no 24 and 26, Table 11, Figure 48J). Both patients were adult male diagnosed as common ALL,
among that one was diagnosed as T-cell ALL. Overall, most del(6q)-positive cases occur in the common ALL subgroup. Only rarely has ALL with del(6q) displayed a mature B-cell phenotype and belonged to the L3 subgroup [Harrison CJ. et al; 2009]. In the present study, both have completed maintenance cycle of treatment protocol so del6q21 associated with good clinical outcome and served as favorable marker as compared to other studies.

Deletion(1)(p32): The most frequent rearrangement in, 16% of T-ALL, leading to over expression of TAL1 is a submicroscopic interstitial deletion of part of 1p32. It involves STIL (SCUTALZ interrupting locus, previously known as SIL or SCL), which lies centromeric of TAL1. These abnormalities leading to high TAL1 expression are common in childhood T-ALL. They are found in 17% of such cases and are associated with a favorable outcome [Harrison CJ. et al; 2009].

In the present study del(1)(p32) observed in one adult male patient (Case no 22, Table 11, Figure 48P). Patient was resistant to treatment so re-induction cycle was introduced. Patient was expired before remission achieved. Thus it was associated with poor outcome, contradictory to literature. That is may be due to presently del(1)(p32) observed in only one patient. So the present result is in contrast to literature.

Dicentric(9;12)(p13;p12): Incidence of dic(9;12) reported approximately 1% in childhood ALL. The breakpoint positions in 9p have been shown to vary considerably at the molecular level, although FISH and molecular studies have shown that the abnormality can give rise to a fusion between ETV6 on 12p13 and PAX5 at 9p13. The PAX5-ETV6 fusion protein acts as an aberrant transcription factor with repressor function, leading to a block in B-cell differentiation [Harrison CJ. et al; 2009].

In the present study dic(9;12)(p13;p12) was observed in 12 year male patient diagnosed as ALL L1 (Case no 1, Table 11, Figure 48E). Patient has completed six maintenance cycle of treatment protocol and was live at last follow up. It is associated with good prognosis.
Importance of FISH in ALL patients

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). FISH is one of the most sensitive molecular methods used to detect genetic abnormalities (Seyed MM. et al; 2012). When cytogenetic studies are normal or insufficient, FISH may detect cryptic rearrangements, rare or slowly proliferative abnormal populations in non-mitotic cells. It helps in detecting known cryptic rearrangements as well as identification of new abnormalities (translocation, duplication and amplification) at the gene level.

In the present study, FISH was performed with probes for BCR-ABL DCDF, AML-ETO DCDF, MLL Break apart, PML-RARα DCDF, and different WCP probes to estimate the incidences of different genetic subgroups with abnormalities involving above genes in ALL, to characterize new abnormalities.

In the present study, out of 37 patients with structural chromosomal abnormalities, FISH assay was performed in 32 patients for BCR-ABL DCDF probe (Table 16). Total 26 patients (case no: 1 to 19 and case no 25 to 31, Table 16, Figure 44) showed typical positive signal pattern i.e. OGFF. Variant signal pattern was observed in 6 patients. Out of 6 patients, 3 patients (Case no: 32 to 34, Table 16, Figure 45) showed signal pattern OGFFF which indicated presence of double Philadelphia chromosome. In the present study, 2 patients were observed with mix clone population (Case no: 35 to 36, Table 16, Figure 46) and one patient with variant signal pattern indicated deletion of 5′ABL gene (Case no. 37, Table 16, Figure 49). In one patient BCR-ABL DCDF probe used to confirm del(22)(q) (Figure 48F). It was also used to confirm numerical chromosomal abnormalities. In one patient (Case no.1, Table 15, Figure 37) trisomy of chromosome 21 was confirmed using AML1-ETO DCDF probe. In two patients (Case no.2, 3 and 4, Table 15, Figure 38) trisomy of chromosome 8 was ruled out using AML1-ETO DCDF probe. In a patient (Case no. 9, Table 15, Figure 39) with trisomy of chromosome 17 was ruled out using PML-RARα DCDF probe. In two patients (Case no. 4 and 8, Table 15, Figure 40) trisomy of
chromosome 8 and 21 were ruled out using AML1-ETO DCDF probe. In one patient monosomy of chromosome 9 was ruled out using BCR-ABL tricolor probe (Figure 43).

In the present study one patient with complex chromosomal translocation (Case no. 40, Table 11, Figure 48R) involving chromosome 1 and 2 were ruled out using WCP 1 (Spectrum Orange) and WCP 2 (Spectrum Green). It was revealed that t(1;?), t(?;2). In Case no. 1, Table no. 20 using WCP FISH; the exchange of chromosomal material between chromosomes 1, 6 and 11 was confirmed. WCP 1 with spectrum orange and 6 with spectrum green which revealed, del(1q) and t(1;?) (Figure 52A). WCP 6 with spectrum orange and 11 with spectrum green confirmed unbalanced translocation (6;11), t(1;11) and add 6(q) with unknown chromosomal material (Figure 52B). Presence of ring chromosome 2 (Case no. 2, Table 18, Figure 56) was confirmed using WCP FISH for chromosome 2. Presence of ring chromosome 9 (Case no. 5, Table 18, Figure 57) was confirmed by using BCR-ABL DCDF.

Present 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.

**Importance of M-FISH in ALL patients**

The development of molecular cytogenetic techniques based around FISH heralded a significant advance in the accuracy of cytogenetic analysis and has provided many tools for research in both clinical and tumour cytogenetics [Speicher and Carter, 2005]. The achievement of 24 colour FISH-based karyotyping (M-FISH, SKY, COBRA) [Chudoba I. et al; 1999] was the result of further technological advances in this technology and has been one of the great successes of molecular cytogenetics in the past decade.

The majority of karyotypes in childhood ALL are abnormal, with approximately 20% of cases having either a 'failed' cytogenetics result, or exhibiting an apparently normal karyotype [Harrison et al; 2005]. Because of the poor morphology of ALL chromosomes, M-FISH may provide a valuable adjunct to karyotyping [L. Kearney. 2006].
In the present study, M-FISH analysis was performed in total three patients. In one patient (Case no. 8, Table 15) M-FISH was used to detect numerical chromosomal abnormalities in hyperdiploid patients. The M-FISH has successfully disclosed the numerical abnormalities involving five different chromosomes i.e. +5, +8, +12, +16 and +21 (Figure 41). In one patient with structural abnormality (Figure 48S), M-FISH was used to confirmed t(11;14)(p14;q21). The cryptic and complex rearrangements involving four chromosomes was unraveled by M-FISH and observed four-way translocation with exchange of chromosomal material between chromosomes 1, 4, 6, and 11 which was beyond the limitation of only GTG banding and WCP FISH (Case no. 1, Table 18, Figure 50B). This case was novel [Patel DM. et al; 2012].

The present study indicates strength of M-FISH is in defining numerical and structural abnormalities in complex karyotypes, thus it helped to characterize the nature of CCR that could not be fully defined by CC.

**Noteworthy cases observed in the present study**

**Novel cases:**

**Case no. 1, Table 18, Figure 50:** 46,XY,t(Y;3)(q?;q?),t(1;4;6;11)(q31;q27;q22;q23)[10]

In this patient, CCR involving chromosomes 1, 4, 6, and 11 were observed with 11q23 rearrangement. These abnormalities have not been reported before, although cases involving 11q23 reported as a recurrent abnormality [Mitelman; 2012]. The gene at 11q23 is reported as the 'mixed-lineage leukemia,' or 'myeloid-lymphoid leukemia,' gene (MLL, also referred to as ALL-1, HRX, or HTRX1). More than 54 reciprocal chromosomal loci are known to participate in 11q23 translocations [Huret JL; 2007]. The most common are 4q21, 9p22, 19p13, and 1p32, and many partner genes have been identified to contribute to the function of the MLL gene. The present case reported novel partner loci 1q31, which may be the crucial event in leukemogenesis, probably resulting from alternative splicing events that need to be further characterized. The involvement of additional chromosomal anomalies of sex chromosome t(Y;3) in addition to t(1;4;6;11) is a unique observation, and this is the first report of t(Y;3) in a patient with ALL [Mitelman; 2012]. Thus, the role of t(Y;3) in ALL leukemogenesis is not known. However, the patient expired during treatment.
Discussion

course. It represent poor prognosis in CCR. It indicates high genomic instability in malignant cells at chromosomal level [Patel DM. et al; 2012].

Case no 2, Table 18, Figure 54: 47,XY,t(5;7)(q;q)+10[3]/47,XY,+10[3]/46,XY[1]

In Mitelman database 832 cases with trisomy 10 chromosome recorded, approximately 33 cases with isolated trisomy 10 have been described. Over two-thirds of cases associated with this cytogenetic abnormality are AML variant in the FAB classification (one-third of cases). Trisomy 10 has been, however, described in all of the FAB variants. One case each of biphenotypic acute leukemia and eosinophilic leukemia with trisomy 10 have been described. Two cases of high grade MDS (i.e. refractory anemia with excess blasts-2 (RAEB-2)) have been associated with isolated trisomy 10. Most of the cases of ALL were of the Pre-B type [Lewis ZT; 2006]. There have been eight cases of ALL with +10 as the sole abnormality and six cases have been reported for t(5;7) in ALL but no case with +10 with t(5;7) is described [Mitelman; 2012]. Hence, this case considered as novel finding.

Case no 3, Table 18, Figure 55: 45,XY,t(6;19)(q16;p13),-21[7]/46,XY,t(6;19)(q16;p13)[1]

In Mitelman database 166 cases with monosomy 21 chromosome recorded, one case with t(6;19)(p21;q13),-21 but no case with t(6;19)(q16;p13) is described. Hence, this case considered as novel finding [Mitelman; 2012].

EPHA7 (EPH receptor A7) mapping at 6q16.1, is a member of the EPH family located in the membrane. The gene involved in ATP Binding, ephrin receptor activity, nucleotide binding, protein binding, receptor activity and transferase activity. It is involved in human colorectal carcinogenesis and not clearly define role in leukemogenesis. TCF3 (also known as E2A) mapping at 19p13, essential for normal B-cell hematopoiesis. The gene is involved in numerous translocations with different partner genes. It forms homodimers and heterodimers with other basic helix-loop-helix transcription factors, such as ASCL1, MYOD1, TAL1, MYOG, NEUROG1, and TWIST1, producing various chimeric transcripts involved in hematological malignancies resulting from different molecular mechanisms. NOTCH3 mapping at 19p13.12 is a membrane receptor that mediates cell-cell interactions to facilitate cell differentiation, growth and cell death. Translocation of chromosome 19p was also
found in several other chromosomes, including chromosomes 12q, 14q, 17q, 4q, and 6q. Over expression of \textit{NOTCH3} full-length mRNA is associated with a 19p translocation. Chromosome 19p13.12 amplification harboring the Notch3 gene is frequently identified in ovarian cancer [Sugimura H. et al; 2007].

**Case no 4, Table 18, Figure 56**: 46,XY,der(2),r(2)[5] and **Case no 5, Table 18, Figure 57**: 46,XX,r(9),t(9;22)(q34;q11.2),der(22)t(9;22)(q34;q11.2)[5]

The incidence of cases with ring chromosomes is generally low in human hematopoietic neoplasias, rather than in ALL it is about 3.4%. Among a consecutive series of 152 childhoods ALL, however, only one case with a ring chromosome was found [Andreasson et al., 2000]. Ring chromosome is often unstable, leading to concomitant genetic loss or amplification. This may explain why ring chromosome is extremely rare in hematopoietic malignancies [Sivendran S, 2010, Masaaki Adachi, 2012].

In the present study among 273 total ALL patients, we have observed 2 cases with a ring chromosome (case no. 4 and 5), a 21 years/male patient with ring chromosome 2 and 65 years/female patient with ring chromosome 9. Literature reported that chromosome 9 were involved in 16% cases and chromosome 2 were involved in less than 1% cases, of all rings formation cases in ALL. In leukemias, for instance, the presence of ring chromosomes seems to be associated with poor prognosis. In some tumor sub entities, they are so frequent and typical that they can even serve as cytogenetic hallmarks for differential diagnosis [Gebhart E. 2008]. In the present study, 0.6 month survival in case no. 4 and disease persistent in case no. 5 indicates shorter survival and poor prognosis, respectively.

In Mitelman database, no case described with der(2),r(2). Hence, the case no 4 considered as novel finding. Till date, total 7 cases with r(9) chromosome have been observed, but no case with r(9),t(9;22)(q34;q11.2) is described. Hence, the case no 10 considered as novel finding [Mitelman; 2012].

**Rare Cases:**

**Case no 6, Table 18, Figure 58**: 46,XX,t(5;13)(q31;q34) [7]/46,XX[2]

In Mitelman database total 4 cases with t(5;13)(q31;q34) reported [Mitelman; 2012].
AF5q31 (ALL1 fused gene from chromosome 5q31) mapping at 5q31, mostly observed in fetal tissues (heart, lung, brain, liver); at a low level in adult tissues; therefore, AF5q31 may play a critical role in the fetal development. It is poorly defined and only 2 cases to date reported. RAP2A mapping at 13q32.1, is a member of the RAS superfamily of monomeric GTPases, closely related to RAS. There are two isoforms, RAP2A and RAP2B that share 90% identity and are encoded by two different genes. RAP2 proteins share 50% identity with RAS proteins, including the regions involved in GDP/GTP binding. Function is clearly unknown [Huret JL; 2003].

Case no 7, Table 18, Figure 59: 46,XY,t(11;15)(p15;q22)[10]
In Mitelman database total 6 cases with t(11;15)(p15;q22) reported [Mitelman; 2012].

Gene NUP98 mapping at 11p15 involved in different types of AML, as fusion gene with HOXA9, DDX10, HOX D13, TOP1, PMX1 and LEDGF. It has 29 known partners for recurrent translocation. It is also involved in T-cell ALL (t(4;11)(q21;p15), either L1 or L2 in the FAB classification) mature T and myeloid markers variably co-expressed epidemiology rare, approximately 10 cases described. Evaluated in 2-5% of adult T-ALL and not evaluated in childhood ALL. Both sex equally involved and generally found in children or young adults, Prognosis probably unfavorable and median survival reported below 18 months [Kearney L; 2010]. In the present study, the patient was lost to follow up during fifteenth day of third maintenance period of treatment protocol after 16.4 months of diagnosis, hence treatment outcome is not evaluated. GCNT3, TP53BP1 and KIAA0101 are located on 15q22. GCNT3 is important in protecting the normal functional architecture of colon epithelial cells. TP53BP1 gene is a protein component of the cellular response to DNA damage. KIAA0101 gene is thought to be oncogenic through modulation of DNA repair pathways via interaction with PCNA. It is not clearly described in leukemogenesis [Joseph S. et al; 2011, Radhakrishnan P. et al; 2007].

Case no 8, Table 18, Figure 60: 46,XX,del(20)(q11)[7]
A rare chromosomal abnormality i.e. del(20)(q11) was observed in a female patient. In Mitelman database total 13 cases with del(20)(q11) reported [Mitelman; 2012].
Discussion

E2F1 is mapping at 20q11.22 expressed in all actively proliferating tissues in a cell-cycle specific manner. It is expressed mainly at late G1 and G1/S transition, and its mRNA is absent or low during the rest of the cell cycle. E2F1 represents a controversial player in cell cycle control, exhibiting a dual behavior, sometimes acting as a tumor-suppressor and others as an oncogene. E2F1 exerts transcriptional control over the cell cycle, induces apoptosis via distinct pathways, and participates in DNA damage response and checkpoint. E2F1 can induce apoptosis via distinct P53-dependent and independent pathways. Due to its pivotal and multifunctional role in cell cycle control, E2F1 is expected to be a significant player in carcinogenesis. Nevertheless, its paradoxical behavior, i.e. acting as an oncogene or a tumor suppressor depending on the cell context, renders its characterization and study challenging. The most usual genetic alteration of the E2F1 gene is amplification, as has been reported in several leukemic (e.g. the HEL human erythroleukemia cell line) and melanoma cell lines. The 20q gains and the amplification of the E2F1 gene are linked with greater aggressiveness and poorer prognosis [Zachariadis M. et al; 2008]. However, present case is representing deletion 20q11 and probably that may be the reason for longer survival. The patient was known to live and in remission at last follow up after 55.2 months of diagnosis.

Case no 9, Table 18, Figure 61: 47,XY,+8,add(13)(p11)[5]

In Mitelman database 771 cases with trisomy 8 chromosome recorded, but only 7 cases with add(13)(p11) reported. So, this is a rare case [Mitelman; 2012].

Trisomy 8 is more often found in B-cell than in T-cell cases. It is a rare anomaly in lymphoid malignancies accounts about 5% of ALL. Rarely found as a sole anomaly (5-10%), may be part of hyperploid karyotypes (>50 chromosomes mainly) without structural anomalies (20% of cases), mostly observed with complex karyotypes with structural anomalies (2/3 of cases), these complex karyotypes being often hyperploid as well and sex ratio is 1.5/1 [Huret JL 2007]. Present case is 9 year/male patient with B-cell ALL.

Gene involved may be FLT3 mapping at 13q11. Expression of FLT3 was described on bone marrow CD34-positive cells, corresponding to multipotential, myeloid and B-
Discussion

lymphoid progenitor cells and on monocytic cells. FLT3 protein is expressed on blast cells from most AML and B-ALL. Mutations in the FLT3 gene are the most frequent genetic aberration that has been described in AML. With 20-25% length mutations in the juxtamembrane domain are the most frequent, followed by 7-8% mutations in the second tyrosine kinase domain, mostly point mutations in codon 835 or deletions of codon 836. Also point mutations in the juxta membrane domain have been described and the number of new mutations all over the total gene is still growing. Internal tandem duplications and/or insertions and rarely, deletions in the FLT3-gene are implicated in 20-25% of all AML. It was also described to be involved in 5-10% MDS refractory anemia with excess of blasts (RAEB 1 and RAEB 2) and rare cases with ALL. An unfavorable impact on prognosis especially a high relapse rate of the FLT3-LM has been shown by many study groups [Schnittger S; 2005]. In present case, probably that may be the reason for that patient expired during fourth day of first induction period of treatment after 0.2 month of diagnosis.

Case no 10, Table 18, Figure 62: 46,XY,t(2;11)(p16;p15)[5]

This rare rearrangement is reported in total 5 cases [Mitelman; 2012]. BCL11A mapping at 2p16.1 contains DNA binding motifs (Zn fingers). When it is over express become oncogene and lead to result in CLL/immunocytoma aggressive disease; possibly also other t(2;14)(p13;q32) in other B-cell diseases (ALL, myeloma) [Huret JL.; 2002]. Gene NUP98 mapping at 11p15 involved in different types of AML, as fusion gene with HOXA9, DDX10, HOX D13, TOP1, PMX1 and LEDGF. It has 29 known partners for recurrent translocation. It is also involved in T-cell ALL (t(4;11)(q21;p15), either L1 or L2 in the FAB classification) mature T and myeloid markers variably co-expressed epidemiology rare, approximately 10 cases described. Evaluated in 2-5% of adult T-ALL and not evaluated in childhood ALL. Prognosis probably unfavorable and median survival reported below 18 months [Kearney L; 2010]. In present study patent was expired before remission achieved. Thus, it indicated poor prognosis.

Case no 11, Table 18, Figure 63: 46,XX,t(8;12)(q21.1;p12)[6]/46,XX[2]

This rare case is a variant of t(12;21). Here, the partner of ETV6 gene is ETO. Till date total 28 partner genes have been identified till date. ETO (eight twenty one) mapping at 8q21.3 it is a putative transcription factor. ETV6 mapping at 12p13 contains a
Helix-Loop-Helix and ETS DNA binding domains with wide expression. It acts as a transcriptional regulator; important in angiogenesis and in bone marrow hemato poiesis. *ETV6* is implicated in leukemia, MDS and sarcoma [Knezevich S.; 2005]. In Mitelman database total 3 cases with t(8;12)(q21.1;p12) reported. Hence, this is a rare case [Mitelman; 2012].

**Case no 12, Table 18, Figure 64: 46,XX,add(14)(q11)[7]/46,XX[4]**

In Mitelman database total 3 cases with add(14)(q11) reported. Hence, this is a rare case [Mitelman; 2012].

The human *TRA* locus is located on the chromosome 14 on the long arm at band 14q11.2. Proteins encoded by the *TRA* locus are the T cell receptor alpha chains, a region that had been described to be involved in translocations. The total number of human *TRA* genes per haploid genome is 116 of which 96 to 98 genes are functional. Total 19 partner genes have been identified till date. Deregulated transcription factors may exert their oncogenic potential by altering the gene expression programs that regulate hematopoietic differentiation of a multipotent progenitor [De Keersmaecker K. et al; 2005].

**Case no 13, Table 18, Figure 65: 46,XX,t(1;22)(p13;q13)[5]/46,XX[7]**

*t(1;22)(p13;q13)* is a rare case in the present study. 60% of cases (mostly patients under 6 months of age) have the *t(1;22)* as a single anomaly; the remaining cases (mainly patients above the age of 6 months) exhibit complex and hyperdiploid clones. Survival was equivalent in cases with or without a complex karyotype; the frequent presence of an additional der(1) indicates that the crucial event is likely to lie on the der(1)t(1;22).

*OTT* (one twenty-two) mapping at 1p13 contains RNA recognition motif consensus and *MAL* (megakaryocytic acute leukemia) mapping at 22q13 involved in chromatin organization may modulate chromatin organization, HOX differentiation pathways, or extracellular signaling [Huret JL. 2001]. In Mitelman database total 6 cases with *t(1;22)(p13;q13)* reported [Mitelman; 2012].
Case no 14, Table 18, Figure 66: 46,XY,t(6;12)(q21-22;p13)[5]
This rare case is a variant of t(12;21). Here, the partner of ETV6 gene is PRDM1. Till date total 28 partner genes have been identified till date [Knezevich S. 2005]. PRDM1 mapping at 6q21 encompasses 23.6 kb DNA in the long arm of chromosome 6. PRDM1 is a transcription repressor that binds to specific DNA sequences through its zinc fingers, and functions as a scaffold for recruiting co-repressors that catalyze histone modifications. It has no known intrinsic histone methyltransferase activity. It has critical role in B and T cells differentiation [Tam W. et al; 2011]. ETV6 mapping at 12p13 contains a Helix-Loop-Helix and ETS DNA binding domains with wide expression. Acts as a transcriptional regulator; important in angiogenesis and in bone marrow hematopoiesis. In Mitelman database total 7 cases with t(6;12)(q21-22;p13) reported [Mitelman; 2012].

Case no 15, Table 18, Figure 67: 46,XX,del(8)(q22)[2]46,XX[5]
This is a rare case with deletion of chromosome 8q22. BAALC mapping at 8q22.3 spans over 90kb with 8 exons. Various transcripts involving different exons giving rise to 5 different proteins. Down regulation of gene give rise to various transcripts found in leukemic blasts. BAALC over expression is associated with a high WBC count and poor prognosis [Huret JL. et al; 2006]. In Mitelman database total 10 cases with del(8)(q22) reported[Mitelman; 2012].

Case no 16, Table 18, Figure 68: 47,XY,+8,t(9;22)(q34;q11.2),i(17)(q10)[8]
An i(17q) has been reported in solid tumors and in various types of hematological diseases: AML and CML, MDS and myeloproliferative neoplasms, ALL and CLL, and Hodgkin and non-Hodgkin lymphomas. It is believed that i(17q) as a sole abnormality is a distinctive clinicopathological entity with a high risk to a leukemic progression. Isochromosome 17q usually occurs at time of blast transformation and heralds an aggressive clinical course. In the 2008 WHO classification system, myeloid neoplasms with isochromosome 17q are only briefly mentioned within the MDS/MPN category. The underlying molecular defect that produces the isolated i(17q) is unknown [Lazarevic VLj. 2012]. In Mitelman database 771 cases of trisomy 8 chromosome recorded, 130 cases of i(17)(q10) reported, and 79 cases of +8,t(9;22)(q34;q11.2)
reported, but only 2 cases of +8,t(9;22)(q34;q11.2),i(17)(q10) reported. Hence this is a rare case [Mitelman; 2012].

**Assessment of morphologic features in present study**

ALL subdivided into three subgroups on the basis of cytomorphologic features (Table 19). The FAB classification [Bennett et al; 1976] constituted the most widely accepted scheme. Although rarely used in recent years, it has provided the basis for the development of current classification systems. The FAB system takes into account both the characteristic morphology of individual cells and the degree of heterogeneity within the leukemic cell population [Harrison C.J. et al; 2009]. In the present study, higher number of patients in ALL-L1 (38 patients) followed by ALL-L2 (26 patients) and ALL-L3 (3 patients) were observed, which is similar to observed by Lilleyman JS. (1986) and contradictory to that reported by Settin et al (2007). Settin et al reported higher number of patients in ALL-L2 followed by ALL-L1 and ALL-L3. In the present study, hemoglobin (8.27 g/dl) levels were higher in ALL-L2 FAB group in accordance to that reported by Venkateswaran SP. et al (2012) in ALL-L2 (8.07 g/dl). However, in present study WBC count were reported higher in ALL-L1 (57.5X10^3/cmm) contradictory reported by Venkateshwaran SP. et al (2012) in ALL-L2 (52.85X10^3/cmm).

Other risk factors and correlation of FAB subtypes have already been established as indicators of disease outcome [Annino L. et al; 2002]. Age is probably the most important prognostic factor. Studies have shown progressively worse outcome for ALL with each advancing decade after infancy [Rowe JM.et al; 2005]. Multiple groups have quoted that overall survival continuously decreases with increasing age [Gökbuget N. et al; 2006, Pullarkat V. et al; 2008] within FAB groups of ALL. In the present study, median age of patients in ALL-L3 was 17 years and the estimate OS was 8.67%. However, in patients ALL-L1 subtype median age was 8 years and OS was 23.19%, thus reflecting the survival disadvantage.

**Assessment of Immunophenotypic features in the present study**

There are several hundred monoclonal antibodies that allow the detection of more than 260 clusters of differentiation (CD) groupings. Complete immunologic
characterization at diagnosis is required to identify subtypes with different presentations and prognosis. Immunophenotyping by flowcytometry has long been considered a critical part of diagnostic evaluation in patients with ALL [Shaikh MU. et al; 2011].

The present study confirmed the close association between T-cell phenotype with male gender, higher leukocyte count, higher platelet count and high hemoglobin level (Table 20) similarly reported by Jaffe ES. et al (2001), Kebriaei P. et al (2003) and Vitale et al (2006). Vitale et al (2006) presented the results of 90 adult patients with T-ALL treated with the GIMEMALAL 0496 protocol. They found a predominance of males (68%) with T-cell ALL, in the present study 60% male were observed which is quite similar. In the present study numerical abnormalities (hyperdiploidy and hypodiploidy) were observed in both Immunophenotype sub-groups. Whereas, Geeta V. et al (2012) reported presence of numerical abnormalities in only T-cell ALL.

**Treatment outcome**

The intensive chemotherapy protocols used in the last 15 years have improved the outcome of treatment of children with ALL. Unfortunately, similar improvements have been achieved only in the more affluent of the developing nations and only in major centers from these countries. Other countries, such as India, have generally reported low survival rates, largely because of limitations in the existing health care systems such that treatment regimens of sufficient intensity are impractical, but possibly also reflecting differences in the general health (nutritional status, intercurrent infections etc.) of the patients or in the biology of ALL [Advani S. et al; 1999].

International opinion is that children with ALL in developing countries have a right to effective treatment [Pui CH. et al; 2004]. However, in many resource-poor settings, chemotherapy drugs are in limited supply and the cost of treatment may be a crippling burden on families. Appropriate curative therapy in developing countries may need to differ from the standard treatment followed in developed countries. Designing such protocols needs an understanding of the local spectrum of the disease.
In the present study, patients were selected based on single induction chemotherapy protocols i.e MCP 841 to abolish cost effectiveness of treatment; a situation frequently encountered in developing countries. Patients with Philadelphia positive were additionally received Imatinib Mesylate.

Several studies on ALL outcome, using primarily uniform initial treatment regimen, describe relapse pattern but do not address treatment and outcome of relapsed ALL [Kulkarni. et al; 2011]. Except as described by Goyal et al (2005), limited therapy has been administered to the most relapsed patients in sharp contrast to intensive therapy and bone marrow transplant employed in resource plenty nations. Based on the currently available data, the percentage of patients with relapsed ALL is likely high in India. In the present study among the 273 included patients, 188 (69%) achieved complete morphological remission (CR) and 85 (31%) patients were not responded to therapy. Of the 188 CR patients, 103 (103/273, 37.7%) patients relapsed. Among the different cytogenetic categories patients, no case reach to maintenance cycle in hyperdiploidy with t(9;22) category. Maximum patients in normal karyotype category completed maintenance cycle of treatment protocol (Table 21).

Estimated 10,000 new childhood ALL cases diagnosed annually. Among them, Outcome of only a small fraction of the expected number of relapsed patients is compiled by Kulkarni KP. et al (2011) in literature, is described in Table 25.

It is currently difficult to estimate the overall post relapse survival for total ALL patients through the entire country. However, it is likely poor due to the limitation of significant resource along with socioeconomic and infrastructural constraints in several centers [Kulkarni KP.et al; 2011].
Discussion

Table 25: Treatment outcome from Indian ALL patients.

<table>
<thead>
<tr>
<th>Authors</th>
<th>a</th>
<th>Age (yr)</th>
<th>Relapse (%)</th>
<th>Isolated</th>
<th>Combined</th>
<th>Timing**</th>
<th>Treated Outcome</th>
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<td>Aiya et al, 2010</td>
<td>254</td>
<td>&lt;15</td>
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<td>532</td>
<td>&lt;15</td>
<td>11* (2.06)</td>
<td>1</td>
<td>1</td>
<td>1 (9)</td>
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</tr>
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<td>407</td>
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<td>30 (7.37)</td>
<td>112</td>
<td>8 (26.7)</td>
<td>6 (20)</td>
<td>13* (23.3)</td>
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<td>16 (53.3)</td>
<td>6 (20)</td>
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<td>-</td>
<td>-</td>
<td>3* (25)</td>
<td>15* (17.5)</td>
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<td>9</td>
<td>7*</td>
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<td>Abstracts</td>
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In all the studies and abstract initial treatment in all patients was primarily a uniform, non-risk stratified therapy regimen (like MCP841 protocol) (Source: Kulkarni KP et al, 2011)

There is clear need of generation of accurate epidemiological data and reporting of all cases with a consolidated nationwide effort. Determination of cytogenetics, molecular and biological characteristics of relapsed disease would be helpful in identifying high-risk features relevant to local population and in advocating risk adapted therapy. With a potentially enlarging cohort of patients with relapse, focus needs to be on therapy and salvage of relapsed disease along with improvements in upfront therapy.

Overall Survival in 273 ALL patients

The most common FAB type among present cases was ALL-L1, which showed the higher survival, ALL-L2 group showed intermediate survival and lower survival for ALL-L3 group (Table 22, Figure 73), contradictory to that reported by Settin et al (2007), a common FAB type was ALL-L2 associated with poor prognosis.

Among the 273 patients with cytogenetic risk groups, the estimated mean OS of favorable risk group was higher and lower for adverse risk group (p<0.041, Table 22; Figure 72).

Previous reports consistently showed that high WBC is associated with higher risk of relapse and poorer OS [Kantarjian H. et al; 2004, Annino L. et al 2002]. This is mostly
true with ALL of B-cell origin [Gokbuget N. et al; 2006]. Most of our patients have ALL of B-cell (80.8%). WBC counts were reported higher in T cell (100x10^3cmm) as compared to B-cell (60x10^3cmm).

Many groups have confirmed the superior outcome of T-lineage ALL as compared to B-lineage ALL [Gökbüget N. et al; 2001]. This is in sharp contrast to present study, where patients in the B-ALL subgroup did fairly well. The estimated mean OS of B-cell ALL (16.36 months) was higher than T-cell ALL (10.73 months) (Table 22; Figure 74). This could be due to the higher number of patients with B-ALL as compared to T-ALL.

However, there was no significant difference in survival among patients with different subtypes of ALL (B and T cell lineage) (p<0.308). This could be due to the insufficient number of patients that are needed for such a comparison.

Like the LALA-94 (Leucémie Aiguë Lymphoblastique de l'Adulte-94 protocol) patients [Charrin C. et al; 2004], patients with hypodiploidy in the present study had a significantly reduced OS and DFS then hyperdiploidy group. The estimated OS of hyperdiploidy group was higher than other cytogenetic categories (23.49% CI, 13.65-33.33 months) and OS of hypodiploidy group was 20.35% (CI, 10.32-30.38 months) (p<0.0001, Table 22; Figure 71). The estimated DFS of hyperdiploidy and hypodiploidy groups were 26.84% (CI, 16.91-36.77) and 15.07% (CI, 10.02-20.14), respectively (p<0.128, Table 24; Figure 78).

However, present study demonstrated that, the adverse effect was independent of age and WBC. The mean age of hyperdiploidy and hypodiploidy were 13.12 years and 17.1 years, respectively (Table 12, Figure 31). The mean WBC of hyperdiploidy and hypodiploidy were 35.6x10^3cmm and 57.1x10^3cmm, respectively (Table 12, Figure 32). However, age was observed high in t(9;22) with hyperdiploid category (37.4 years) and WBC count was higher in miscellaneous category (86.5x10^3cmm) among all cytogenetic categories. These observations are consistent with previous studies which have examined the prognosis of these ploidy subgroups separately [Secker-Walker LM. et al; 1997, Charrin C. et al; 2004]. These patients had no other
adverse features, such as high WBC or increasing age; hence, cytogenetics represents the only method of identifying them.

The results of present study emphasized the importance of cytogenetic studies in the evaluation of ALL, especially in view of the particularly poor outcome of Miscellaneous category (OS 12.86%) and t(9;22) with Hyperdiploidy category (OS 1.40%).

**Comparison of overall Survival in coexistence of favorable and poor prognostic cytogenetic markers**

Another finding of the present work, those patients with additional abnormalities with Ph positive patients had shorter OS. A recent report from the Japan Adult Leukemia Study Group (JALSG), patients treated with imatinib combined chemotherapy supported our results [Yanada M. et al; 2008], but some other studies, in pre-imatinib era, showed no significant effect of additional aberrations [Wetzler M. et al; 2000, Faderl S. et al;1998]. So, the significance of additional aberrations in Philadelphia positive ALL patients should be further investigated in the Imatinib era with large cohort.

Rieder et al (1996) detected hyperdiploidy with Philadelphia positive ALL in 17% patients, who achieved complete remission more readily than t(9;22), although the duration of remission and overall survival was similar in the two group. Thomas et al (1998) have also suggested an improved outcome for Philadelphia positive ALL patients with the hyperdiploid karyotype compared to those with other karyotype abnormalities although the differences in survival did not approach significance. Both the studies have indicated that, the genetic heterogeneity of Philadelphia positive ALL that could be potentially translate in to variable outcomes.

Contrasting results have been obtained from studies on the prognostic implications of hyperdiploidy in adult Philadelphia positive ALL by the groupe Francais de cytogentique Hematologique [groupe Francais 1996] in large series of 433 patients with ALL, 11 of total of 127 patients with Philadelphia positive ALL chromosome also had a high-hyperdiploid karyotype, but the outlook for Philadelphia positive with hyperdiploid patients did not differ from those without hyperdiploidy. A similar lack
Discussion

of benefit of the high hyperdiploid karyotype in adult Philadelphia positive ALL was also suggested by Ribera et al (2002).

In the present study observed shorter survival for hyperdiploidy with t(9;22) than t(9;22) (p<0.0001, Table 23, Figure 75), there was also survival difference between hyperdiploidy with t(9;22) and hyperdiploidy group (p<0.002, Table 23, Figure 76). This is presumably by altering the kinetics of Philadelphia positive neoplastic cells indicates that genetic heterogeneity of Philadelphia positive ALL that could potentially translate in to outcome. However, present study did not find significant association between t(9;22) and hyperdiploidy group (p<0.313, Table 23, Figure 77).

Contrasting results obtained in the present study from which have been reported by other study groups showed that diverse cytogenetic changes with favorable and unfavorable prognosis suggest various mechanisms of leukemogenesis in ALL. Thus, based on the outcomes from the present study, along with some reports from the literature, it is conceivable that the simultaneous presence of additional karyotypic abnormalities like Philadelphia positive and hyperdiploidy may alter the biological properties of cells and influence the clinical outcomes. In addition, it is one of the most common mechanisms of resistance to imatinib is the mutation involving the ABL kinase domain, and secondary aberrations in Philadelphia positive ALL patients. It may be associated with the genes instability, which facilitates the occurrence of mutation [Schultz KR. et al; 2010]. It may partially explain why inferior OS was detected in patients with additional aberrations with Philadelphia positive ALL in the present study.

The present study showed predictive and prognostic factors in patients with ALL in a research center in a developing country. Significant prognostic factors in our treated group were age, WBC count and platelet counts, while single chemotherapy regimen was used. Factors described earlier in the literature to affect survival such as cytogenetic abnormalities, and cell lineage were significant in present study. Hence, all the above limitations and the relatively low socioeconomic status of Indian population, the OS (18.53%) and DFS (20.31%) of patients enrolled in the present study were quite lower than the international data [DeVita VT. et al; 2011].