Discussion
AML is a heterogeneous group of disorders that can be sub-typed by morphology as well as immunophenotyping, cytogenetic, and molecular techniques. Chromosome aberrations account for approximately 52% of all de novo AML. Many chromosomal aberrations in AML are non-random and are closely associated with specific FAB cytomorphological subtypes. Several chromosomal and genetic abnormalities that have been extracted from analysis of prognostic factors for AML are recognized as important in selecting treatment strategies. They are useful in the classification of AML and required to establish the disease entity. Cytogenetic features are important aspect prognostic factors in AML. WHO classification also includes cytogenetic and genetic features for diagnosis and management of AML patients.

Non-random chromosomal abnormalities can be detected in the majority of cases of acute leukemia using classical high-resolution banding techniques. These abnormalities include balanced reciprocal chromosomal translocations, such as t(8;21)(q22;q22) or t(15;17)(q22;q21); internal deletions of single chromosomes, such as 5q- or 7q-; gain or loss of whole chromosomes (+8 or -7); or chromosome inversions, such as inv(3), inv(16), or inv(8). Complex chromosomal abnormalities are observed in approximately 15% of de novo cases that do not have an antecedent hematologic disorder; this constitutes a clinical group of patients with particularly poor prognoses [DeVita VT et al., 8th Ed.]. Numerous recurrent karyotype abnormalities have been discovered in AML. Till 2004, total 10718 different aberrations, 126 recurrent aberrations, 86 different genes and 97 fusion genes were recorded [Mitelman F et al., 2004]. This number was increased up to 58,145 in May 2010 [Mitelman 2010].

Total 321 patients including 194 (60.4%) males and 127 (39.5%) females were enrolled in present study. All patients were categorized in to three different cytogenetic risk groups based on their chromosomal abnormalities. Moe Wakui [2008] has shown that approximately 30% of patients have recurrent genetic abnormalities. In present study we found 34 % (109 of 321) of the patients with recurrent genetic abnormalities. Despite substantial information on chromosome
abnormalities in AML, the role of ethnic and geographic variability in chromosome abnormalities of patients with acute leukemia has been poorly described in only a limited number of reports [Greenlee RT et al., 2001, Wingo PA et al., 2003, Ries lag HD et al., 2006]. Objective of the present study was to determine the karyotypic pattern of de novo AML patients from the racial Indian population. This is the first systematic cytogenetic report on AML in Gujarat, India. Several studies have reported a high incidence of t(8;21) in AML [Jemal A 2002, Aquino VM 2002].

**Studies on etiology:** AML is the most common type of leukemia in adults, yet continues to have the lowest survival rate of all leukemias. AML accounts for approximately 25% of all leukemias in adults in the Western world, and is most frequent Worldwide. Newly diagnosed AML patients have a median age of 65 years [Forman D et al., 2003]. AML in adults has a male predominance in most countries [Forman D et al., 2003]. The incidence of AML is somewhat higher in males and in populations of European descent [Jabor EJ et al., 2006]. Incidence rates for AML have been reported to be nearly stable over time among the different age groups. There was slight increase noted among the oldest group. In the present study the median age was 27.5 years which is less than observed in the literature. Male predominance was observed in present study.

t(15;17)(q22;q12-21) and t(8;21)(q22;q22), are the two most common reciprocal translocations in both adults and older children with AML and have never been detected in infants aged less than 12 months. However, the incidence of t(15;17) [and inv(16)(p13q22)/t(16;16)(p13;q22)] is similar in adults and older children, t(8;21) is twice as common in pediatric as in adult AML [Mrozek K et al., 2004]. In accordance to the report by Mrozek [2004], present study showed that the patients with t(15;17) and inv(16) were observed in the age range from 5-65 years and 2-45 years.

**Cytogenetic Risk group:** It has long appreciated that diagnostic cytogenetic is one of the most valuable prognostic indicators in AML. Multiple recurring
chromosomal aberrations have been identified, and in many instances, genes altered by these aberrations have been mapped and cloned [Mrózek K et al., 2000]. Most commonly found abnormalities include t(8;21), t(15;17) and inv(16), del5q, del7q, 11q23 rearrangements. In the present study, all patients were classified into favorable, intermediate, or adverse risk groups based on karyotypes according to results of the AML MRC 10 trial [Grimwade D et al., 2009]. The favorable risk group included patients with t(8;21), t(15;17) and inv(16), whether alone or in combination with other abnormalities. The intermediate risk group included abnormalities like normal karyotype, patients with t(9;22), 11q23 rearrangements and other structural and numerical abnormalities. The adverse risk group included patients with a complex karyotype with four or more numerical or structural aberrations, -5, deletion(5q), and -7, whether alone or in combination with intermediate risk or other adverse risk abnormalities [Oh 2008]. The identification of favorable, intermediate, and adverse chromosomal/genetic aberrations has become increasingly informative in AML patients, and is used to stratify these patients into different treatment modalities [Ahmad F. et al., 2008]

**Chromosomal abnormalities in AML patients in favorable group:** First chromosomal abnormality in favorable group comprises t(8;21) with and without chromosomal abnormalities. t(8;21) were frequently associated with additional chromosomal aberrations which is one of the most recurrent abnormalities in AML. Approximately 3-4% of AML patients associated with t(8;21) have variant translocations [Vundinti BR et al., 2008]. Secondary changes includes del(9)(q) and loss of X chromosome and loss of Y chromosome in 12.7%, 16.3% and 39.3% respectively [Bakshi SR et al., 2004, Mrozek K et al., 1997]. Leukemia characterized by del(9q) may follow a similar pattern. Schoch et al [1996] reported that the additional aberration of del (9q) in t(8;21) leukemia imparts an unfavourable outcome. Significantly shorter median Overall survival was observed as compared to patients with t(8;21) with or without loss of a sex chromosome [Slovak M et al., 2000]. In the current study, only 1 patient documented del(9q) and 19.6 months
survival was observed, definitive conclusions could not be drawn; for its response to treatment, as only 1 patient was found.

There is continuous debate regarding significance of loss of sex chromosome in patients with t(8;21). A large study showed that the loss of Y chromosome is associated with aging, not with hematological diseases. In another study, loss of Y chromosome was associated with hematological disorders [Zhang JL et al., 2007]. Different studies were carried out to find out that frequency of Y chromosome is related to malignant change or it is age related phenomenon. The reports showed loss of Y chromosome at initial diagnosis but a normal 46,XY karyotype during remission which proved that it was disease related phenomenon. Zhang JL et al [2007] also showed that the frequency of Y chromosome loss is significantly higher in patients with hematological disorders than in patients without hematological disorders. It indicates that the loss of Y chromosome is associated with a neoplastic change. In the early studies, the loss of Y chromosome in bone marrow cells was reported in elderly males with or without hematological disorders. It has been demonstrated that normal males start to lose the Y chromosome in bone marrow cells at age of 60 years [Wiktor A et al., 2000]. Loss of Y chromosome as a sole abnormality or with additional cytogenetic changes has been reported in AML, although it is not clear whether or not this abnormality is a marker for the leukemic clone. Studies have supported the theory that loss of Y chromosome is not a phenotypic event associated with the aging process in males. In present study, sole t(8;21) was found in 19 (42.2%) patients (Table 9). Loss of sex chromosome was found in 44.4% (20 of 45 patients) (Table 10) of patients which is quite comparable with earlier reports [Zhang LJ 2007]. In accordance to literature, loss of sex chromosome was found in 11.11% (5 of 45) of female was also very less. Loss of Y chromosome was observed in 3 patients with older age (Table 10, patient 9, 10 and 19) in present study. Previous data from our laboratory documented, that lost Y chromosome was reappeared as patients achieved remission after therapy and during clinical remission [Bakshi SR et al., 2004]. The patients included in the present study were of the lower age group than that was observed in the literature.
Thus present results indicate that the sex chromosome loss may relate to neoplastic clone rather than to aging process. Further, large studies are required for better understanding of the role of sex chromosome loss in hematological neoplasia. Also loss of Y chromosome could provide a proliferative advantage simply because it tends to replicate late in S-Phase. Its loss might therefore shorten the cell cycle slightly [Van DD et al., 2001].

Trisomies for chromosomes 4 and 8 are observed in 6-10% of patients with t(8;21). Additional cytogenetic abnormalities, irrespective of their nature or complexity, do not appear to have a deleterious effect on remission, relative risk of death and overall survival. At relapse, additional chromosomal abnormalities include rearrangement of the long arm of chromosome 7, deletion of the long arm of chromosome 9, gain of chromosome 8, and on rare occasions the duplication of der(21)t(8;21).

**Trisomy 4** is a rare nonrandom cytogenetic abnormality found in association with AML [Gupta V et al., 2003]. Oh investigated that approximately 30% of the patients have recurrent genetic abnormalities [Oh 2008]. A few cases of AML, namely, one M2 and five M5 have been described in which trisomy 4 was part of a complex abnormal karyotype with both numerical and structural anomalies of other chromosomes. The Mitelman database for chromosomal aberrations in cancer queried for trisomy 4 with t(8;21) in AML showed only 17 different cases [Mitelman 2010]. Rarely gain of chromosome 4 or chromosomes 10 are reported as the sole abnormality in AML. Trisomy 4 occurs in AML with frequency of <1% and a strong association with the presence of double minutes has been described. In the present study, a patient with trisomy 4 with t(8;21) was observed (Table 1, patient 2). There was no presence of double minutes in all metaphases observed. CD56 expression in AML is reported in granulocytic sarcoma and multidrug resistance, and is known to confer poor prognosis in AML-M2 with t(8;21) and APL. The patient was lost to follow up within few days; hence the CD56 status was unclear. Therefore, relation between CD56 expression and trisomy 4 needs further investigation. While
development of AML with trisomy 4 secondary to chemo or radiotherapy has also been suggested. In the present study, patient had no history of long-term medication, radiotherapy, or any relevant occupational exposure. The present case of AML-M2 is a rare case in terms of cytogenetic results. Even though trisomy 4 is likely to be a secondary event after t(8;21) translocation, the presence of this additional numerical aberrations may define a distinctive subtype [Trivedi PJ et al., 2009]. Follow up of more such cases over a period of time is required to know the possible prognostic effect of this cytogenetic entity.

Frequency of trisomy 15 in AML is very rare and it has marked male predominance and found mostly in adults. Prognosis and genes involved in +15 is still not known. Trisomy 15 as a sole chromosomal anomaly was also reported in ALL, MDS, CLL, Hodgkin disease, non Hodgkin lymphoma, anaemia, thrombocytopenia and dyserythropoiesis. Trisomy 15 has been noticed as a sole chromosomal anomaly in patients with AML. Smith et al [1999] estimated the incidence of trisomy 15 in AML at 0.33%. Smith et al and Sinclair et al proposed that the possibility of the trisomy 15 abnormality is linked to ageing, because most hematologic neoplasia with trisomy 15 were elderly males. Also 14 out of 30 patients showed a concomitant abnormality of a missing Y chromosome [Sinclair et al.,1998, Sinclair et al. 1999]. Baumgartner et al., [2000] demonstrated a possible association between loss of sex chromosomes and trisomy 15 might have certain biological significance rather than accidental concomitance. Missing sex chromosomes are encountered in AML patients with t(8;21), but trisomy 15 is very rarely found in this type of leukemia [Suzuki A et al., 2001]. In the present study, trisomy 15 was observed in a male patient with younger age and no loss of sex chromosome along with t(8;21) (Table 11, Patient 1). The trisomy 15, might be disease related phenomenon. Till date only 13 cases are found with trisomy15 associated with t(8;21) in AML-M2 subtype meaning that it is a recurrent abnormality [Mitelman 2010].

In the present study, a female patient with i(7)(q) together with t(8;21) was observed (Table 11, Patient 3). To the best of our knowledge, till date there was
not a single case was noted with i(7)(q) in AML-M2 [Mitelman 2010]. So this is the first novel case of i(7)(q) in AML-M2. Patient expired, within 1.5 months so the prognosis of patient with this additional change might be very poor. More cases should be accumulated and studied to find out its significance in AML. There might be presence of very harsh genes present on this region and isoformation of this gene cause over expression of these genes which might have playing role in leukemogenesis.

The association of loss in 7q with myeloid leukemia suggests that this region contains a tumor suppressor gene or genes whose loss of function contributes to leukemic transformation or tumor progression. In the present study, a male patient showed t(7;7)(p21;q22). Till date there was only two cases with t(7;7) so this is a rare translocation [Mitelman 2010].

Besides involvement in the AML associated t(8;21), AML1 has other known gene fusion partners. Some rare partners include breakpoints at chromosomal bands 1p36, 5q13, 12q24, 14q22, 15q22, 17q11.2, 16q24, 18q21 and 19q13.4. These well documented occurrence of AML1 gene rearrangement in chemotherapy induced secondary AML and secondary MDS is most likely with topoisomerase II inhibitors and cytostatic drugs [Athena M Cherry et al., 2001]. Translocations involving 21q22 are commonly observed in both de novo and therapy-related AML and MDS. They often result in the disruption of RUNX1 and give rise to fusion genes consisting of RUNX1 and different partner genes, which contribute to leukemogenesis. To date, at least 21 such translocations are known from the literature [Dai H et al., 2007]. Till date variant translocations of t(8;21) involving chromosomes 1, 2, 4, 5, 6, 7, 8, 10, 12, 13, 15, 17, 18, 19, or 20 have been reported, with a frequency of ~3.4% [Kokate P et al., 2008]. Patients with variant translocations of AML1 gene showed poor prognosis. Till date 21, different partners of AML1 gene have been identified. Identification of variant AML1 related translocations provide short cut for identifying new genes associated with leukemogenesis [Dai H et al 2007]. It is possible, therefore, that the rearrangements in patients might have result in the disruption of
the \textit{AML1} gene and its fusion to unknown partner genes, at 1q12 with the possible production of two fusion proteins. Candidate partner genes for \textit{AML1} at 1q12 include \textit{ARNT} and \textit{MLLT11} (alias \textit{AF1Q}). In the current study a female patient (Table 11, Patient 4) karyotype result showed 46,XX,t(1;21)(q22;q22)[8]. The patient was diagnosed as having AML-M2, with no history of chemotherapy or radiation exposure. Thus, it belongs to de novo AML. Findings indicate a reciprocal translocation between chromosomes 1 and 21, which resulted in disruption of the \textit{RUNX1 (AML1)} gene on chromosome 21. Although, the involvement of chromosome 1 has been reported in two \textit{AML1}-associated translocations, their breakpoints were located at 1p32 and 1p36 respectively. It was found that chromosomal regions 1q12 have not previously been reported to be involved in \textit{AML1}-associated translocations. FISH results verified disruption of the \textit{AML1} gene. It was found that \textit{AML1} (\textit{RUNX1}) gene was situated on 1q. Therefore it was concluded that t(1;21)(q22;q22) is a novel chromosome translocations involving the \textit{AML1} gene.

\textbf{Second most commonly observed chromosomal abnormality in favorable group t(15;17):} APML characterized by the presence of t(15;17)(q22;q21). Translocation which causes the fusion of the retinoic acid alpha gene (\textit{RARa}) to the promyelocytic leukemia gene (\textit{PML}) located on 17q21 and on 15q22 respectively. The two chimeric genes, \textit{PML-RARa} and \textit{RARa-PML}, are thought to play a role in leukemogenesis. A small proportion of patients with APML have complex or simple variants of this translocation [Abe S et al., 2008]. Chromosomal rearrangements in addition to t(15;17) have been reported in 25 to 40\% of APML. The most common secondary changes observed are trisomy 8, ider(17), del(9q), and del(7q) [Kim M et al., 2005]. Trisomy 8 as secondary changes in patients with APML and most frequent abnormality, accounting for about one third of the additional abnormalities comprises 26\% to 39\%, of the secondary changes.

In the present study, t(15;17) and additional changes were observed in 58 patients. Mainly involved breakpoints were del(1)(q), add(4)(q), add(8)(q), add(5)(q) and
add(7)(q). The complete hematological remission was observed in 30 patients and no remission was found in 10 patients whereas 18 patients were lost to follow up. Out of 58 patients 8 (13.8%) patients expired during the study period, 12 (20.6%) patients were alive with disease remission and 41(70.6%) patients were lost to follow up. Comparison of clinical characteristic and outcome of APML patients with or without additional chromosomal changes to the t(15;17) was carried out. Conventional karyotyping on banded metaphases has shown that, in addition to the specific t(15;17), 30% to 35% of APML patients have additional cytogenetic changes, most frequently trisomy 8. In APML, trisomy 8 appears to have little or no impact on prognosis [Wolman SR et al., 2002]. In present study total 8(13.8%) patients (Table 13) were with secondary changes from that 3(5.1%) patients were with trisomy 8 which was quite less as compared to reported cases. In the patients with secondary changes, out of 8 patients, 2 patients died during the course of treatment. 4 patients were lost to follow up and 2 patients were alive during the study.

I(17)(q10) and ider(17): Structural aberrations involving chromosome 17 are frequently observed in human carcinomas. According to the Mitelman database [Mitelman 2010], i(17q) can be noted in approx 2.5% of hematologic malignancies [Bown et al., 1999]. In a prospective clinical trial of patients with AML aged 60 yrs and above, abnormal 17p and 17q were among the most frequent findings (82%–100%) with complex karyotypes [van der Holt et al. 2007]. Babicka et al. [Babicka et al., 2006] showed that the 17p11 is the only region that has been frequently affected and having a pathogenic role during progression and clonal evolution in different hematological malignancies. The 17p11, particularly the 17p11.2 sub-band, is a highly genetically unstable region and presents a unique genomic architecture, marked by several dispersed as well as adjacent and both directly oriented and inverted low-copy repeats [Carvalho CMB and Lupski JR. 2008].

In formation of i(17)(q) the actual order of events could not be confirmed, the possibility of the presence of a cell line with a single sub microscopic insertion of PML into RARA on a normal chromosome 17 suggested that the i(17)(q) evolved
secondary to this event. The patients responsiveness to ATRA appears to be similar to that of classical APML cases with the t(15;17) [Lee GY et al., 2005]. Since the t(15;17) affected both arms of the isochromosome, it is logical to assume that the translocation occurred first and the formation of the isohromosome later or, both events might have taken place simultaneously. In the present study, i(17)(q10) were found in 2 patients (Table 13, patient 7 and 8) at diagnosis as a secondary change. Mechanism behind isoform involved loss of p arm and duplication of q arm as shown in Figure 65.

*Figure 65: Mechanism of i(17)(q10).*

Duplication of rearranged q arm form ider(17)(q). ider(17)(q) was found in 3 patients in one male patient and one female at diagnosis (Table 13, patient 2 and 6 and) and 1 female patient (Table 12, patient 41) at relapse and both female patients expired, one female after one year of diagnosis and the other female within a week after diagnosis. Present study could make a distinction between ider(17)(q) from i(17)(q10) by different types of FISH PML-RARA probes. With the help of two different LSI PML-RARA probes, it was established that ider(17)(q10) show duplication of RARA-PML fusion. 1st event is t(15;17), second event is deletion of p arm of der(17) and third event is rejoining of fusion RARA-PML on p arm of der(17), this phenomenon form duplication of RARA-PML fusion (Figure 66).
Current cytogenetic data strongly suggested two separate clonal evolution events. 1st is formation of isochromosome 17 and after that the involvement of the chromosome 15 and 17 in translocation. In other words, two clonal pathways took place; one involving chromosome 15 and 17, and the other involving chromosome 15 and the isochromosome 17. A correlation between isochromosome 17 and a mechanism of disease progression has not yet been established. Loss of a copy of the TP53 tumor suppressor gene on chromosome 17p is an important mechanism, associated with tumorigenesis. The other mechanism, associated with isochromosome is that the oncogenes THRA and ERBB2 on chromosome 17q are duplicated and their protein products are amplified. Numerous investigators have insisted that, deletion of p arm cause loss of TP53 gene and this leads to reduction of the total p53 level. This phenomenon, disrupts the integration of genetic repair and apoptosis may be interfered with and that this can contribute to disease progression. In contrast, several studies insist that one homolog of 17p is normal and thus any direct link of TP53 inactivation and progression is unclear [Kim M et al 2010]. Patient with ider(17)(q10) tends to have shorter survival, when it presented at relapse. It suggests that it might be an additional independent poor prognostic factor, still prognostic importance need to be studied in more similar cases. Combination of use of cytogenetics and FISH using different probes in more cases can improve understanding of additional genetic events that contribute to leukemogenesis.
**Trisomy 10** is a rare non-random cytogenetic abnormality found in association with AML. The hematological and clinical features associated with this finding have not yet been clearly defined. Some authors reported as a minority component of a more comprehensive AML study and therefore lacking a full description of both clinical and hematological features [Llewellyn IE et al., 2000]. In the current study, trisomy 10 (Table 14, Patient 1) was observed as disease progressed. At diagnosis patient’s cytogenetic result was non informative whereas FISH result was PML-RARa fusion was positive. After 2 years, this patient relapsed and showed clonal evolution with trisomy 10 and FISH result was PML-RARa fusion was positive. Patient was lost to follow up, so further clinical information was not obtained. A subset of APML (29-43%) showed other chromosome aberrations in addition to t(15;17) either at diagnosis or relapse but the influence of atypical phenotypic, cytogenetic or molecular features present at different stages of the disease, on the clinical outcome is still unclear. However, some of the chromosomal aberrations were found to be associated with atypical morphology and/or drug response, indicating a genotype-phenotype correlation [Kurian S et al., 2002]. Chromosome 10 is the mapped location of a number of genes of possible significance in cancer. These include the **BMI1** gene on 10p13, the **est** gene on 10p11.2, the **RET** proto oncogene on 1q11.2, the **HOX 11** gene on 10q24, and the **MX1** gene on 1q24-25. Although these genes may be related to the leukemic phenotype, it is also possible that trisomy 10 is a secondary event resulting from genomic instability in the transformed cells [Estalilla O et al., 1998]. A literature review revealed 13 cases of trisomy 10 in AML and 7 cases were observed in AML-M3 subtype. So this is a rare chromosomal abnormality.

**add(8)(q):** Association of add(8)(q) in APML is still unclear. It is assumed that genes within the duplicated region might be activated as oncogene due to of duplication of particular arms. **C-MYC** gene is situated on q arm of chr. 8 so there might be over expression of gene dosage. An accumulation of similar cases and the characterization of those cases will help answer the question in the future. In the current study, it was found that add(8)(q) together with t(15;17) (Table 14, Patient 8). To the best of our knowledge, not a single case of add(8)(q) with
t(15;17) is recorded in the Mitelman database [Mitelman 2010]. Thus, this is the first case of add(8)(q) with t(15;17).

**Trisomy 21:** Though cases of APML in Downs Syndroms have been described in the medical literature rarely, it is rarer still to find the microgranular variant (M3v) of APML in trisomy 21 patients. It differs from other subtypes of AML in that the patients are often younger. 90% of children with AML have no known risk factors, a number of predisposing constitutional disorders have been found in the remaining 10% of children [Jain D et al., 2007]. In the present study patient was with younger age but not having Down syndrome, so trisomy 21 might be clonal evolution due to disease progression.

**Variant translocations with t(15;17):** A small number of APML cases lacking t(15;17) has been described, including three simple variant translocations, PLZF-RARA fusion in t(11;17)(q23;q21), NUMA1-RARA fusion in t(11;17)(q13;q21), and NPM1-RARA in t(5;17)(q32;q21). Complex variant translocations, especially three-way translocations, are increasingly recognized. Complex variant translocations occurred between chromosomes 15 and 17 and one or more other chromosomes. Complex variant translocations have been increasingly reported in APML. At least 33 cases with three way complex translocations have been reported to date. Several recurrent translocations have been reported in APML showing three way or more complex rearrangements, among these translocations, recurrent breakpoints identified include 1p36, 2q21, 3p21, 4q21, 11q13, 19p13, 20p13 and Xq13. Each of these breakpoints has been found in two to three cases but no gene was identified as a putative oncogene. In terms of morphology and treatment outcome, most of these cases did not differ from APML with the typical t(15;17) [Chryssa S et al; 2008].

In the present study, a patient (Table 14, Patient 5) showed 46,XX,t(5;15),t(7;15),t(15;17). Patient was treated with ATRA and chemotherapy showed a good response as of typical APML. The role of involvement of another chromosome in APML is still unclear. Further studies would be required to clarify the
clinical features and prognosis with complex translocations [Shori A et al 2008]. This karyotype result was an example of one step mechanism established with the chromosome and FISH results. 1st translocation between chromosomes 15 and 17 took place to generate a t(15;17), followed by splited PML gene from \textit{RARa-PML} fusion was translocated to chr.5 and chr.7, producing t(5;15), t(7;15) and t(15;17). This patient is still alive in hematological remission with ATRA therapy. To the best of our knowledge, our case is the first report of a complex variant translocation with t(15;17) involving chr. 5 and chr.7.

**T(5;17):** In the present study, a male patient showed t(5;17) which was a variant of t(15;17) (Table 14, Patient 9). Conventional karyotype result with G banding showed 46,XY,t(5;17)(q?q;ql2)[10]. With metaphase FISH result using LSI \textit{PML-RARa} it was observed that there was 2R3G signals which indicated that there was no \textit{PML-RARa} fusion but signals of \textit{RARa} were situated on chr.5. So in the current result, partner of \textit{RARa} was \textit{NPM} gene not \textit{PML}. Therefore, present case emphasizes the usefulness of metaphase FISH along with interphase FISH.

**Third chromosomal abnormalities in favorable group-Inv(16):** Inv(16) is one of the third karyotypic abnormalities seen in AML that are associated with a favorable outcome. It included patients with inv(16) with and without additional chromosomal abnormalities in favorable group. Presence of inv(16) in AML is assoicated with more favorable prognosis. Deletions of the 5' region of the \textit{MYH11} gene have been detected in ~20% of patients with inv(16). A correlation between this deletion and survival has not been confirmed in other reports [Egan N et al., 2004]. Standard karyotypic analysis remains the “gold-standard” for the detection of cytogenetic aberrations. However, both inv(16) and t(16;16) may be subtle, cryptic, or masked by deletions and thus difficult to detect using standard cytogenetic techniques, especially in metaphase spreads showing suboptimal chromosomal morphology [Merchant SH et al., 2004].
In G banded karyotype results 3 patients showed del(16)(q). Out of the 3 patients, FISH analysis with CBFB/MYH11 probe was possible in only 1 patient and showed CBFB-MYH11 fusion and inv(16) was confirmed. Present study highlights the importance of FISH in detecting small deletions in association with AML-M4 and inv(16).

Cryptic rearrangements involving chromosome 16 [inv(16)(p13q22), del(16)(q22) and t(16;16)(p13;q22)] are frequent findings in AML. These rearrangements can occur as the sole karyotype change or in association with additional chromosomal abnormalities, including trisomy 22 and deletion of the long arm of chromosome 7. Trisomy 22 is considered as rare and a non random secondary chromosomal abnormality associated with inv(16) in patients with AML [Litmanovich D et al., 2000]. Trisomy 22 is an uncommon karyotypic aberration in AML and is often associated with inv(16)(p13q22) [Lu CM et al.2006].

Trisomy 22 in AML-M4 should always alert to the presence of an inversion 16. This cryptic rearrangement is difficult to identify by even the most experienced cytogeneticists. Inv(16) seems to be a prerequisite in AML-M4 and is associated with a favorable response to conventional multi agent chemotherapy[Xu W, 2008]. Till date mitelman database of chromosome aberrations showed only 14 cases with trisomy8, trisomy 22 with inv(16) [Mitelman 2010].

**Intermediate group chromosomal abnormalities:**

This group is very heterogeneous because it includes patients with a normal karyotype and so-called other chromosome aberrations, most of which have a poorly defined or debated prognostic impact. Intermediate group included patients with normal karyotype, t(9;22), other numerical and structural abnormalities.
The most common trisomies, in decreasing order of frequency in de novo AML are, +8, +22, +13, +21 and +11. Trisomy 22 is a non-random secondary aberration accompanying inv(16)/t(16;16) and is rarely seen as the only chromosome abnormality. Numerous trisomies at diagnosis, present as a secondary aberration i.e. +8, +13, +11 and +21. These trisomies are observed recurrently and found in de novo AML as 4% for sole +8, 1% each for sole +13 and +11, and 0.4% for sole +21. With regard to the impact of recurrent trisomies on clinical outcome, most data have been gathered for trisomy 8, but results have been somewhat inconsistent.

**Normal karyotype:** Patients with a normal karyotype are the single largest cytogenetic subset of adult AML. Normal karyotype represents the largest cytogenetic subgroup (40%-50%) of AML. They are classified in the intermediate prognostic category, because their complete remission rates, CR duration and survival probabilities are usually lower than those of adequately treated patients with t(8;21), inv(16) or t(15;17), but higher than patients with unfavorable chromosome aberrations. However, this group is highly heterogeneous at the molecular level, and is likely to be composed of subsets with varying prognosis. A small fraction of patients determined to have a normal karyotype by conventional cytogenetic analysis may carry gene fusions typically associated with recurrent translocations or inversions. Occasionally, this happens because the presence of a chromosome aberrations is not recognized only by the cytogenetic approach, especially when the quality of chromosome preparation is suboptimal and the missed rearrangement subtle, e.g., t(11;19)(q23;p13.1), t(15;17), or inv(16). However, in other patients, the gene fusion results from a cryptic rearrangement involving segments smaller than the length of a single band and thus unrecognizable by standard cytogenetic analysis. It is also rather unlikely that a large proportion of cytogenetically normal patients with de novo AML harbor hidden aberrations detectable only by FISH-based techniques [Mrozek 2004]. Approximately 45% of adults younger than 60 with de novo AML have normal cytogenetics [Claudia DB et al., 2003]. In present study normal karyotype was observed in patients with 1-75 years. Incidence present study
was 36.4 % (117) of patients with normal karyotype which is fairly less than literature [Claudia DB et al., 2003].

**T(9;22):** Translocation 9;22 was found in only 1% of newly diagnosed AML patients. There are some controversy that patients with t(9;22) represents a true acute leukemia or it is features of CML with blast crisis (CML-BC). But several reports have suggested that additional cytogenetic aberrations like extra copies of ph and trisomy 8 are not common in philadelphia positive AML. The t(9;22) cytogenetic abnormality be added to the group of poor cytogenetic risk factors in AML [Soupir CP et al., 2007]. In the present study, there were 5% (16 of 321) patients with t(9;22). Present results showed significant number of patients. There was not a single case with +8, or extra copy of ph chromosome with t(9;22), so this results confirmed that this chromosomal abnormality was of intermediate group of AML and not CML-BC. In the current study, only out of 16 patients with t(9;22), 4 patients expired within one month of diagnosis.

**11q23 rearrangement (MLL rearrangement):** Cytogenetic aberrations in the chromosomal band 11q23 and rearrangement of MLL gene are important prognostic factors in AML. Translocation 11q23 in AML is associated with unfavourable prognosis [Schoch C et al., 2003]. Structural abnormality of the 11q23 band (11q23) bearing the MLL translocation is a recurrent chromosomal change observed in 3% to 4% of AML patients and is more frequent in younger subjects with de novo (5%-7%) AML or with t-AML (10%-15%) evolving after chemotherapy. It is rarely observed in older patients (60 years or older), with AML [Cox MC, et al., 2004]. The incidence of MLL rearrangements in AML-M4 and AML-M5 were around 10% to 30%, but its frequency in non-M4-M5 AML is unclear. Poirel H et al [1996] observed that six (20%) of the 29 AML-M1 had MLL rearrangements, but did not found its incidence in M2, M3, M6 and M7 subtypes. AML-M3 may rarely have the gene abnormality, at least at diagnosis.
In the present study, 11q23 was found in 6.1% (12 of 197) patients from the intermediate group and 3.7% of all 321 patients, which was similar to the observations from literature. As described in literature, it was found mainly in younger patients, which was quite similar. In present study, 7 patients with less than 15 years age and 5 adult patients with 36-65 years age range. As it was observed in only 1 patient with 65 years, in present study, it was similar to literature that it was rarely observed in older adults. Age range of present result was 2-65 years. Patients with AML-M1 showed higher frequency i.e. 7 patients. 3 patients with AML-M2, only 1 patient with AML-M4 and 1 patient with AML-M5 subtype and not a single patient with AML-M3, AML-M6, and AML-M7 subtype was observed.

Till date, more than 100 different chromosomal translocations in association with MLL rearrangements and at least 64 fusion partner genes of MLL rearrangements have been identified and reported in the literature. Among these, five MLL rearrangements, accounting for ~80% of all MLL translocations bearing leukemias, are most frequent t(4;ll)(q21;q23), MLL/AFF1 (MLLT2), t(9;ll)(p22;q23), MLL/MLLT3 (AF9); (11;19)(q23;p13.3), MLL/MLLT1 (ENL), t(10;ll)(p12;q23), MLL/MLLT10 (AF10); and t(6;11)(q27;q23), MLL/MLLT6 (AF6) [Park TS et al., 2009].

In pediatric AML, the main translocations are t(9;11), t(11;19); t(6;11) and t(10;11) accounting for almost 15-20% of the cases [Balgobind BV et al., 2009]. Mainly involved breakpoints in MLL rearrangements were chr.6q, 9p, 10p, 17q and 19p in present study. A recently published series showed that 25% of MLL rearrangement cases were missed by conventional cytogenetic analysis and several of these cases had insertion of the MLL gene. AF10/CALM is a non-random translocation described in AML, ALL, and non-Hodgkin lymphoma. FISH analysis with MLL probe gives more information of MLL partners. Status of MLL gene should be determined only after confirmation with FISH analysis. This statement was also supported by Slovak [Slovak ML et al., 2000] that, patients with karyotypically normal AML represent a heterogeneous population. Caligiuri et al also detected sub microscopic duplications of the MLL gene in about 10% of karyotypically normal AML patients [Slovak ML et
al., 2000]. Not infrequently, reciprocal MLL translocation appears at the chromosomal level as an unbalanced rearrangement and is referred to as add(11)(q23) or del(11)(q23). In the present study, conventional cytogenetics was combined with FISH analysis. Use of commercial probe permits the identification of all MLL rearrangements and translocated partner chromosomes. In present series, FISH with LSI MLL breakapart probe permitted the revision of 4 of 12 cases. Results showed that, del(11)(q23) was revised to i(11)(q), t(11;19)(q23;p13), t(6;11)(q22;q23), t(9;11)(p;q23) and t(10;11)(p12;q23), t(11;17). Hence the current study also emphasized the role of FISH in 11q23 rearrangements.

The prognostic effect of 11q23 abnormalities might depend on the partner gene. Several studies have shown that 11q23 abnormalities with t(6;11) and t(10;11) are associated with a poor prognosis. Whereas, t(9;11) is associated with a superior overall survival and might respond well to intensive treatment, especially when the chemotherapy regimen includes high-dose cytarabine.

**T(1;11):** Till date, there are only 8 cases noted with t(1;11) in AML-M2 subtype. However breakpoint which was observed in present study was not previously reported, so this is a novel case. [Mitelman 2010].

**T(6;11):** The t(6;11) translocation can escape recognition with conventional banding techniques and thus may well have been described in the past as a simple long arm deletion of 11 i.e. del(11)(q23). FISH techniques have proved a useful tool for identifying the translocation. Survival analysis for the patients with AML and t(6;11) indicated that their prognosis was poor, although this result can only be confirmed by studying a larger group of patients [Tamai H et al., 2008]. Among several MLL rearrangements involving chr.6, t(6;11)(q27;q23)(MLL- AF6) is the most commonly found. In addition t(6;11)(q21;q23) has been reported frequently in AML. Two mostly observed t(6;11)(q13;q23) and t(6;11)(q15;q23) are the most rare type of MLL rearrangement. Involving long arm of chr.6. Only two cases with t(6;11)(q13;q23) and three cases with t(6;11)(q15;q23) have been reported.
Recently t(6;11)(q13;q23) has been reported. Genes present in 6q region encode a key mediator of apoptosis. In addition, deletion of 6q15~q16.1 was reported as possibly contributing to the definition of a molecularly defined high risk group. Tae Sung Park [2009] showed that AF6 gene abnormality including MLL/AF6 rearrangement seems to be related to poor clinical outcome [Park TS et al., 2009]. In the present study, conventional cytogenetic result showed del11q23, however with LSI MLL breakaprt probe, it was confirmed that MLL split orange signal was situated on 6q. In present study, patient showed loss of sex chromosome with del(11q23). FISH results revealed that this was a t(6;11)(q;q23). This patient was lost to follow up after diagnosis so further cytogenetic and clinical details were not available.

**T(9;11):** Earlier reports indicate that patients with t(9;11) have a relatively more favorable prognosis than those with other partner chromosomes or genes [Schoch C, 2003, Dohner K, 2002, Byrd JC, 2002, Mitterbauer-Hohendanner G, 2004]. The MRC and the Southwest Oncology Group classify the risk for patients with t(9;11) as intermediate and poor, respectively. Even more disagreement surrounds the prognostic relevance of classic t(9;11) vs variant 11q23(v11q23). Results of different clinical trials showed that patients with t(9;11) have better prognosis than patients with v11q23, whereas other studies failed to identify differences. These discrepancies reflect that 11q23 aberrations are heterogeneous. Furthermore, because many v11q23 translocations are rare translocations, the clinical impact of specific single variants is difficult to extrapolate, even from large studies on 11q23 [Cox CM et al.,2004]. In the present study, t(9;11) was found in 10 years old female patient. With conventional cytogenetics, it was not possible to identify t(9;11) but FISH techniques with LSI MLL break apart probe showed t(9;11)(p?q23). The patient achieved complete hematological remission and was lost to follow up after six months of diagnosis. Further additional information regarding disease was not found. Therefore, it can be assumed that t(9;11) might have better prognosis.
**T(10;11):** CALM/AF10 is an interesting leukemic fusion for several reasons: a) it is found in several subtypes of leukemia and in lymphoma; b) quite often the t(10;11) is the sole cytogenetic abnormality in leukemias suggesting an important role for CALM/AF10 in the development of the leukemia. It is one of the recurrent rearrangement and majorities of patients achieved complete hematological remission [Lillington DM et al., 1998]. The most common breakpoint seen on chromosome 10 was band p12 [Lillington DM et al., 1998]. The number of cases in current study was very small as there was only 1 patient observed. Also, the t(10;11) translocation appears to define a subset of younger patients in 11q23 rearrangement. DM Lillington [1998] noted that there were adult patients in their study and they all were male. Also in the present study, there was a male patient with 36 years of age and with AML-M4 diagnosis which is in accordance to study of DM Lillington [1998]. CALM-AF10 and MLL-AF10 are two translocations that are indistinguishable by conventional cytogenetics. These recombinations may be cryptic, associated with normal karyotypes, or masked in complex karyotype. In the present study, it was possible to identify presence of MLL gene on 10p only by FISH with LSI MLL probe but exact region of 10p was still unclear. So present study highlight that molecular studies are necessary to distinguish which genomic rearrangement is produced, to ensure accurate molecular characterization, and to correlate genetic findings with morphologic and immunophenotypic features, in order to classify leukemias within specific hematologic-genetic entities.

**T(11;19):** The translocation t(11;19)(q23;p13) is well documented in the literature with 111 cases cited in the Mitelman database in all subtypes of AML whereas found only in 20 cases of AML-M2 subtype [Mitelman 2010]. This translocation has been reported in a wide variety of hematological malignancies but the majority have been found in ALL or AML with M4 or M5 type. The breakpoint on chromosome 11 is consistently at band q23.2. However; the breakpoint on chromosome 19 is variable and can occur at either p13.1 or p13.3.3–5. Both the translocations, t(11;19)(q23;p13.1) and t(11;19)(q23;p13.3), are discernible by standard banding analysis. However, due to the morphology of chromosome bands on 19p, each is
more likely to be detected by a particular banding technique. The t(11;19)(q23;p13.1) translocation is readily identified by reverse banding but difficult with Giemsa or quinacrine banding. The converse is true for the t(11;19)(q23;p13.3) translocation. These two translocations involve the same gene, MLL, on 11q23 but different genes on 19p13: ELL at 19p13.19, and ENL at 19p13.3 [Moorman AV,1998].

t(11;19)(q23;p13.3) was found in AMLL and also in some times AML and biphenotypic leukemia also occurred so this translocation is heterogeneous. The association between t(11;19) and FAB type M4 or M5 is consistent with 11q23 abnormalities as a whole, with 80% of 11q23 patients having a M4/M5 FAB type. Both types of translocations are associated with poor prognosis. Still there is evidence that a subset of patients with t(11;19)(q23;p13.3) may have a good prognosis. The apparently good survival of these patients with an 11q23 abnormality remains a paradox. Previous study by AV Moorman et al [1998] found that out of six patients five patients were male. In the current study, a male paediatric patient with AML-M2 subtype was observed with t(11;19)(q23;p13.3), that is similar to the literature and might be related to poor prognosis. Also t(11;19) was related to any particular gender should be determined with large patients group. This was a recurrent chromosomal abnormality. Patient showed complete hematological response and alive.

**T(11;17):** In majority of cases, t(15;17) was observed with APML diagnosis. However, recently several alternative translocations have been reported in APML, including t(11;17)(q23;q12), t(5;17)(q35;q12) and t(11;17)(q13;q12) . t(11;17) has been observed in 3 cases of Acute monocytic leukemia and it involves the MLL gene located at 11q23. In addition to the RARα gene located at 17q12 the MLL gene has also been shown to partner with at least 3 other genes within long arm of chr.17. t(11;17) is recurring abnormality. The association of both PML-RARα and PLZF-RARα fusions with the APML phenotype argues for a key role for RARα in the hybrid genes. It is also possible that PML and PLZF have an equivalent function. The PLZF gene is a
putative transcription factor that appears to be associated with myeloid differentiation and may be deranged in other myeloid malignancies. The identification of PLZF justifies those molecular studies of other APML variant translocations should be systematically performed to determine if genes other than PML (or RARα) may be involved in these APMLs (McConnell MJ and Licht JD 2007). In the present study, there were two patients (Table 20, Patient 3 and 7) with AML-M1 diagnosis showed t(11;17)(q23;q22). However, there was not a single patient with APML diagnosis. In the present study, both patients were male patients. So whether it was related to particular gender remained to be studied with large spectrum of patients. From two patients, one patient was expired during the therapy and one was lost to follow up.

**Other (Numerical and structural) abnormalities in intermediate risk group:**
Numerical abnormalities have been reported more frequently than structural abnormalities in AML, the frequency being about 58% in some studies. The high frequency of different numeric or structural abnormalities affecting the same chromosomes in different clones supports the hypothesis that these karyotypically unrelated clones originate from the common malignant clone through sub microscopic molecular genetic changes and evolutionary processes [Han JY 2006]. In the present study, numerical abnormality was found in 26.4% of the analyzed metaphases and was random. The frequency of numerical abnormalities observed were monosomy, trisomy and tetrasomy of particular chromosome. Most frequently observed chromosome was chr.8. It was observed 14 times. Trisomy 21(X7), +10(X4), +13(3), +19(X3), +4(2), +12, +15, +20(X1). Monosomy X (X2) and 16 (X1).

**Trisomy 10:** Trisomy 10 is a recurring chromosomal abnormality and found in 0.5% only. In the present study it was found as sole in 2 female patients with 38 years of age and 45 years, respectively.
**Trisomy 19:** Trisomy 19 is frequently encountered in cases of CML as a secondary abnormality. However, trisomy 19 rarely occurs as a sole chromosomal abnormality and, to date, it has only been reported in 48 hematopoietic malignancies, 1 case of adenocarcinoma and 1 case of astrocytic tumor [Jung Soon IL et al., 2008]. Present case was the sole trisomy 19 in AML. The 19q13 region is gene rich and includes the AKT2, cyclin E, and MLL2 genes, among others. These particular candidate genes have been implicated in solid tumors and are under investigation in hematologic malignancies as well. Dastugue [2002] study provides further support for investigating the role of chromosome 19 abnormalities in the megakaryoblastic leukemias. Abnormalities occurring during clonal evolution might also be disease specific and help to characterize the cytogenetic profile of a specific malignancy [Dastugue N et al., 2002]. In the present study, two patients showed trisomy 19 and with adult with AML-M1 subtype. As both patients were lost to follow up after diagnosis, further clinical information was not available.

**Trisomy 21:** Cytogenetic abnormalities are frequently associated with various types of leukemia. In particular trisomy 21 is one of the most frequent numerical abnormalities occurring in human neoplasm and is associated with AML, MDS, and ALL, while an additional chromosome 21 is frequently observed in childhood ALL. Acquired trisomy 21 as the sole karyotypic abnormality has been observed in only 0.3-0.8% of human neoplasms, with a lower rate for lymphoid than myeloid malignancies. It is extremely rare karyotypic abnormality in myeloproliferative diseases. Children with Down syndrome (trisomy 21) have a 20 times higher risk of developing AML. Cortes et al reported that 3.3% of the patients with untreated AML or MDS correlated with poor prognosis [Odagak T et al., 2001]. In the present study, total 3(0.9%) patients showed trisomy 21. Of that, there were 2 males and one female. One male patient showed trisomy 21 as sole and one with complex karyotype. Two male patients expired after 13 days and 55 days, respectively. Therefore, it confers poor prognosis.
**Trisomy 13:** Trisomy 13 is a rare recurring chromosomal abnormality. Till date, only four cases of primary acquired isolated tetrasomy have been described in patients with undifferentiated AML [Mitelman Database 2010]. Responses to intensive chemotherapy in older patients (below 60 years of age) are lower than with younger patients, and all described cases of isolated tetrasomy 13 observed in elderly subjects. Silva FP [2007] reported that +13 was observed in older male patients with the average age of 64 years, including 29 males and 7 females. In accordance with literature, both the patients in the present study, were old-aged (60 and 68 years) males. It has also been suggested that trisomy 13 is a poor prognostic factor in acute leukemia. Study suggested that short terms of complete remission were generally seen in these kinds of patients. It has been reported that patients with platelet counts <100X10^9/L survive longer as compared with patients with platelet counts of >100X10^9/L; the platelet count of patient 2 on admission was 35X 10^9/L. It has also been suggested that trisomy 13 is frequently seen in truck drivers, heavy equipment operators or cigarette smokers. From present study, we found 2 patients with trisomy 13 as a sole abnormality. From 2 patient, one patient was an electrician and a chronic smoker and expired within 1 week of diagnosis. Patient 2 was lost to follow-up. The features of Indian patients in acute leukemia with trisomy 13 are not known. To the best of our knowledge, no case with trisomy 13 as a sole abnormality has been reported earlier in India. More cases will be necessary to understand the correlation of trisomy 13 in neoplastic progression [Trivedi PJ et al., 2009].

**Trisomy 8:** Normal cytogenetics constitutes the single largest group in AML. However, several non-random chromosomal abnormalities are also frequent, of which trisomy 8 is the most common numerical aberration (12%) as either a sole abnormality (4%) or part of more complex karyotypes (8%). Recent reports have suggested that AML patients with trisomy 8 have poor outcome and are not responsive to cytarabine-based therapy. Although, some studies have reported that trisomy 8 confers an independent prognostic risk in AML. A German AML cooperative group study reported that prognosis in the presence of trisomy 8 appeared to be
dependent on the other associated clonal cytogenetic changes. In APML, trisomy 8 appears to have little or no impact on prognosis. The clinical impact of additional copies of chromosome 8 on leukemic progression and response to therapy remains controversial. Another attempted explanation of the biologic significance of the extra chromosome was based on the observation of increased copies of the \textit{C-MYC} oncogene that is localized to 8q. Jennings et al., [1998] suggested that increases in \textit{C-MYC} were important to the course of the disease and that trisomy of chromosome 8 was an alternative means for achieving amplification of this gene. It is evident that the molecular and biologic consequences of this trisomy require further investigation. Observations of different studies, suggest that patients with trisomy 8 leukemia have poor overall survival. Mainly for patients with additional unfavourable cytogenetic changes [Wolman SR et al., 2002]. Conversely, in comparison patients with normal cytogenetics with +8 as the sole aberration or patients with t(15;17) with or without +8, no statistically significant differences were found. However, these comparisons suffer from small sample sizes.

The question as to whether +8 is a chromosomal aberration likely to be critical in initiating or promoting leukemia, or whether +8 occurs after the development of leukemia and without an important contribution, is currently unanswered [Virtaneva K et al., 2001]. Trisomy 8 as a Additional Chromosomal abnormalities might have role in generating the factors leading to a poorer risk score still to be find out [Cervera J. 2010].

\textbf{Add(15)(q):} It is not clear whether the genes within the duplicated region were activated as a result of the duplication. An accumulation of similar cases and the characterization of those cases will help answer the question in future [Zhang L et al., 2002]. In the present study, a female patient with AML-M4 subtype was treated with standard induction therapy and achieved complete hematological response.
Adverse group chromosomal abnormalities: This group included patients with normal cytogenetics, 11q23 rearrangements, t(9;22), and other numerical and structural abnormalities.

Complex Karyotype: Complex chromosomal aberrations (CCAs) can be detected in a substantial proportion of AML and MDS patients, de novo as well as secondary or therapy-related, and are associated with an adverse prognosis [Limbergen VH et al., 2002]. Approximately 10% to 20% of patients, with karyotype are complex [Mrózek K et al., 2006]. Patients with complex chromosomal aberrations at diagnosis have overall poor prognosis and respond poorly to chemotherapy. Although, various chromosomes can participate in complex chromosomal rearrangements, those most frequently involved are chromosomes 5, 7, and 11. Cytogenetic and molecular cytogenetic studies have shown that genomic losses or gains are much more frequent than balanced rearrangements in this subset of AML. Among the imbalances most frequently found are losses affecting 5/5q (-5/5q-), -7/7q-, -17/17p-, -18/18q-, 12p-, and -16/16q--; and gains affecting 8/8q (-8/-8q), -21/-21q, ---11q, and-22 [Rucker, FG et al., 2006]. The most common aberrations of chromosome 5 are deletions of 5q, including the 5q31 region, and translocations of 5q to various translocation partners. In the present study, in patients with complex karyotype showed del(5q), t(5;11) and patients,

Total 9 (2.9%) patients were observed with complex karyotype, of the 9 patients 5 patients were of older age which was in accordance with reported cases [Mrozek K 2008]. Most commonly observed breakpoints in complex karyotype in present study was del(5q), 6q, add(11p), add(?7p), del(Xp), del(8p), add(9q), i(17q), del(1q), del(2q), der(4p), add(4q), del(12)(p), del(15)(q), add(11)(p), der(19), add(9)(q), del(5)(p) which was comparable with observations of Rucker FG [2006]. Some novel and rare translocations found in the current study were t(1;6), t(5;11), t(1;19), t(1;3), t(7;12). Numerical abnormalities in complex karyotype was +der(11)t(11;?), +6,+21 i(17q)+21,+6,+8,+19,-20+8,+8,+10. Most frequently observed was trisomy 8 and trisomy 21. Out of 9 patients, one 65 years male patient (Table 22, 163
patient 1) was expired after 2 months which indicated that complex karyotype showed poor prognosis in older patients. It is believed that numerical abnormalities might be results of over expression of gene dosage and translocations might be reflecting genomic instability.

**Tetrasomy 8:** FISH results are in agreement with other previous reports, suggesting that tetrasomy 8 is always accompanied by trisomy 8 clones. Several studies suggested that tetrasomy 8 could occur by either of the following mechanisms: (i) two consecutive events of single nondisjunction of chromosome 8 or (ii) a single event of double nondisjunction of chromosome 8. According to Juwon Kim et al [2008] the first mechanism is more likely, because most reported cases showed that tetrasomy 8 was accompanied by trisomy 8 clones. In addition, the polysomy cases detected by conventional karyotype results should be confirmed by more sensitive tests such as FISH to ensure the possible existence of other clones that may contribute to the aggressive nature of the disease. AML with tetrasomy 8 is considered to have a highly aggressive nature, and the overall median survival has been estimated at 6-7 months by some reports. Recently, Cho et al., [2002] reported a hexasomy 8 in a patient with AML-M5, who also showed a short survival. The pathogenetic mechanisms are not clear. However, genes that may be involved in leukemogenesis located on chromosome 8, such as MYC in 8q24, MOS in 8q22, and RUNX1T1 should be considered as potential causes of malignant transformation. More studies are needed to investigate this rare numerical abnormality in hematological malignancies [Kim J et al., 2008]. In the present study, tetrasomy 8 was found in a male patient with older age who was expired within 2 months of diagnosis. So it can be assumed that tetrasomy 8 also have poor prognosis.

**Abnormalities of 3q rearrangements:** Chromosomal rearrangements involving band 3q26.2 are associated with myeloid malignancies and associated with an unfavourable prognosis and an aggressive clinical course. The 3q26.2 rearrangements are characteristically heterogeneous and typically difficult to detect in poor quality metaphases Abnormalities of 3q rearrangement showed poor
prognosis [Babadilla D et al., 2007]. It is postulated that t(3;8)(q26;q24) is a non random process. In the present study, rearrangement of 3q was found in two male patients with AML-M1 diagnosis. Large group study helps to find out that such rearrangements are male predominance and associated with specific subtype as observed here. One patient was lost to follow up and one showed complete hematological response. This was a very small numbers of patients, study with large numbers of patients are still to be needed to find out correlation of FAB subtype and frequency of involvement of specific gender.

**Monosomy 7:** Loss of chromosome 7 (-7) or del(7q) is a recurring chromosome abnormalities in myeloid disorders. The association of -7/del(7q) with myeloid leukemia suggested that the second region encompassed a distal part of band 7q22. These regions contain a novel tumor suppressor gene(s) and the entire band 7q31 and were defined by the MDS/AML. Loss of such function of such genes contributes to leukemic transformation or tumor progression [Fischer K et al., 1997]. -7/del(7q) is found in only 4% to 5% of paediatric patients with acute myeloid leukemia (AML). The annual incidence of -7/del(7q) is estimated to be 0.3 cases of AML per million children younger than 15 years of age. Patients with-7 and del (7)(q) have been reported to an adverse prognosis [Hasle et al., 2007]. In accordance to observations of Hasle [2007], in present study monosomy 7 was observed in 3 male patients. One patient with AML-M1 subtype and rest of two patients with AML-M2 subtype (Table 23, Patient 1, 2, 3). All three patients were paediatric with age range 4-6 years. Only 1 patient with AML-M2 showed complete hematological response.

**Del(5)(q):** Partial deletion of the long arm of chromosome 5, del(5q), is the cytogenetic hallmark of the 5q-syndrome, a distinct subtype of MDS refractory anemia (MDS-RA). Deletions of 5q also occur in the full spectrum of other de novo and therapy-related MDS and AML types, most often in association with other chromosome abnormalities. However, the loss of genetic material from 5q is believed to be of primary importance in the pathogenesis of all del(5q) disorders [Jaju RJ
1999]. There was only 1 patient with del(5q), so definite conclusion cannot be drawn with such a small data.

**Comparison of overall survival between paediatric and adult AML patients in terms of hematologic response:** It is reported that, the clinical outcome of AML in paediatric patients has improved considerably using intensive chemotherapy and/or stem cell transplantation [Zwaan CM and Kaspers GJ. 2004]. This leads to cure in 50-70% of patients, and also results in significant morbidity and mortality. More than 15% of adults with AML (about 25% of those who attain complete remission) can be expected to survive 3 or more years and may be cured. Remission rates in adult AML are inversely related to age, with an expected remission rate of greater than 65% for those younger than 60 years of age. It was suggested that though remission attained, duration of remission may be shorter in older patients. Increased morbidity and mortality during induction reported to be directly related to age [Dr. G. Quade 2002]. However, in the present study, overall survival rate of both the groups was similar. These results suggest that, there is still a need of customized therapy for better survival of AML patients.

**Comparison of hematological response for overall survival of patients with three different cytogenetic risk groups:** AML patients were categorized into favorable, intermediate and adverse or unfavorable risk group depending on the karyotype abnormalities observed. Several large randomized trials have shown that the projected 5 year survival rates were estimated at 55-65-% for favorable risk group patients, 35-40% for intermediate risk group patients and 10-15% for adverse risk group patients With such drastic difference in outcome, pre-treatment cytogenetics is widely accepted as one of the most important prognostic determinants in newly diagnosed AML [Chin Cheng Chen et al., 2007]. In the present study, results were comparable to the literature. Patients with adverse risk group have very less survival as compared to other two risk groups. This suggest that patient with adverse risk group are associated with poor prognosis and very short survival.
**FISH:** FISH probes capable of detecting the entire length of individual chromosomes (WCP probes) have become a valuable and widely used tool for identifying large number of structural and numerical abnormalities, as well as cryptic abnormalities beyond the resolution of G-banding. It has previously been established, however, that WCP probes have a limited resolution, and fail to detect small chromosomal segments, such as those involved in subtle translocations with breakpoints located in the terminal band [Reid A et al., 2001]. FISH is becoming popular in the diagnosis of clonal chromosomal abnormalities. The speed of FISH is very valuable in AML-M3, because *PML/RARA* positive patients require specific therapy. In the present study, FISH screening in complex or suboptimal quality chromosome and specific FISH analysis for 5q, 7q, 12p, 17p, inv(16), t(11q23) in order to implement CBA accuracy [Cox MC et al., 2003]. FISH proved to be very efficient in detecting trisomies of different chromosomes i.e. 8, 10, 11, 12, 13, 15, 17, 19, 21, 22 and monosomy of 7, 17, 18 and sex chromosomes. Sensitivity of FISH technique is greater than conventional cytogenetic analysis in detecting minor trisomy clones both in those with normal karyotype and in patients with chromosomal anomalies. Different trisomies and monosomies can be identified with FISH probes. The role of FISH in the cytogenetic follow-up of trisomies in AML appears to be promising, especially in view of the lack of more sensitive molecular genetics methods for the detection of aneuploidy. Interphase FISH has also been consistently shown to be a specific and sensitive method for the study of numerical changes in AML. This molecular cytogenetic method possesses greater sensitivity than conventional cytogenetic analysis. Systematic analysis of such cases by FISH would be useful to estimate the incidence of additional chromosomal changes associated with t(10;11) and molecular studies are necessary to elucidate the underlying genomic events [Starza RL et al., 2006]. Numerical and structural chromosome aberrations as detected by FISH on interphase nuclei will in the future become an important additional technique to support and complement the classical karyotyping technique [Poddighe PJ et al; 1991].
The FISH technique can identify isolated abnormal cells among a large group of normal cells and is therefore used for quantitative analysis to monitor the hematologic response to treatment. Another advantage of FISH is that it can detect subtle translocations like t(15;17) which cannot always be detected by conventional cytogenetics [Madon P et al., 2002]. In the present study, most of the breakpoints involved in MLL rearrangements were confirmed with the help of FISH with LSI MLL break apart probe. In the present study, a female patient (Table 20, Patient 1) showed 11q23 and del(17). It was assumed that there might be t(11;17) but MLL probe result showed it was i(11(q) and no cryptic rearrangement with chr.17q. Also t(1;21) ,t(5;17)an t(5;15)(7;15) were identified with the help of different FISH probes only. These translocations were very subtle that could not be identified with conventional G banded metaphases. In the present study, interphase FISH helped to detect different translocations like PML-RARα fusion. Such patients were benefited to therapy and showed complete response [Table 14 patient 5 and 9]. However, the present study highlighted the use of metaphase FISH only as it was possible to differentiate i(17)(q10) from ider(17)(q) with different LSI FISH probes only. Also rare t(5;17)(q33;q12) and variant t(5;15)(q34;q21), t(7;15)(q32;q21) of t(15;17) were identified with metaphase FISH only. In adverse group patients, patient (Table 22, Patient 2) with complex karyotype showed 3 marker chromosomes, that was found as pentasomy 22 with M-FISH but FISH with BCR-ABL showed deletion of BCR gene from 4 copies of chr.22. Such abnormalities were not possible to rule out with conventional cytogenetics. Present study shows that, in addition to the conventional cytogenetic study, FISH study provides further confirmation of chromosomal rearrangements. This facilitates our understanding of the neoplastic process more precisely for the better prognostification of the patient. Primary cytogenetic investigation provides the knowledge and information about the known/unknown chromosomal rearrangements. While FISH study provides further confirmation of these arrangements and also information on minor cytogenetically undetectable rearrangements. This gives information on gene-gene fusion and abnormal clonal formation. Commercially available FISH probes are useful in routine practice when only recurrent structural arrangements are to be studied. Major limitation of FISH is
that only one abnormality can be tested in each hybridization experiment, making karyotype definition impractical with this technique. This limitation has been partially overcome by modifications in the methodology that significantly reduce the costs and may permit the introduction of this method in routine screening for the detection of numerical abnormalities. It is highly sensitive for trisomy but less sensitive for chromosome loss. FISH technique with different probes illustrates results of only targeted chromosome or gene. Whereas, conventional cytogenetic analysis can detect the presence of virtually all chromosomal abnormalities including multiple lesions in a single test. Together, the combination of cytogenetic and FISH analysis incorporates the screening potential of cytogenetics with accuracy of molecular genetics technique. It has lead to better understanding of neoplastic disease, more accurate diagnosis, stratification of patients into genetic subgroups and eventually improvements in treatment and its outcome [VinSheth FJ et al., 2002].

**M-FISH:** Complex chromosomal aberrations can be detected in a substantial proportion of MDS and AML, which are associated with very poor prognosis. Conventional cytogenetics cannot accurately define the specific alterations in complex chromosomal aberrations. M-FISH allows the comprehensive identification of complex chromosomal aberrations [Xu W et al., 2010, Bakshi SR 2009]. According to Smadja et al., [1992] molecular cytogenetic methods are essential components of genetic analysis of patients with complex chromosomal aberrations. New specific chromosomal aberrations with potential diagnostic value have been revealed. It is significantly important to find new recurrent chromosomal rearrangements and breakpoint sites, which might lead to the discovery of new genes involved in origin and progress of malignancy. Numerical abnormalities of chromosome 11, such as trisomies or tetrasomy, reflect additional copies of the *MLL* gene [Braekeleer MDe et al., 2005]. In this study, M-FISH was used in 5 patients. The chromosomes most often involved were 1, 5, 7,8,10, 11, 21, 22, 17, 8 and 19. Derivative chromosome 5 and 1 were implicated most often. The other derivatives were der(11), der(7). M-FISH could refine conventional cytogenetic analysis, find or correct the missed or misidentified aberrations by limitation of conventional cytogenetic analysis. M-FISH
revealed complex rearrangements of chromosome 5 in two patients with in present study. There were t(5;11) which was not found out with conventional cytogenetics. In both the cases, the long arm of the chromosome 5 was translocated to the short arm of the chromosome 11 and 5q31 region was deleted in one case and in other case only loss of long arm of chr.5. The extent of the deletion of 5q is variable; 5q12~5q14, and 5q31~5q33 bands are the most frequently deleted. 5q deletion as a sole aberration suggests good prognosis in patients with myeloid malignancies, but complex rearrangements together with 5q31 deletion is signs of a poor prognosis. Previous reports from our lab also showed that M-FISH is useful in identification of marker chromosome in different malignancy [Bakshi SR 2006, Bakshi SR 2008]. In one patient (Table 21, Patient 2), marker chromosomes were identified as 3 extra copies of chr.22. t(7;12) was possible to identify out with M-FISH technique only. G banded karyotype results could not reveal this cryptic translocation. Also t(8;11) was found only with M-FISH. In conventional cytogenetic result there was addition of 11p only and chr.8 was not found to be affected. Also a patient with complex karyotype showed 3 marker chromosomes that were found as pentasomy 22. Also t(3;17), which was not found in conventional cytogenetics and showed monosomy 17 was identified as t(3;17) with M-FISH results only. The present study demonstrates that in order to understand complex chromosomal aberrations it is crucial to combine conventional karyotyping with molecular cytogenetic techniques. Our findings confirmed that M-FISH was a powerful molecular cytogenetic tool to characterize complex karyotypes in AML.

Follow up patients: The commonly used chemotherapy protocol for AML is daunorubicin or idarubicin for 3 days and cytarabine (ara-C) at a dose of 100 mg/m2 daily for 7 days as a continuous infusion, a regimen commonly known as “3+7” [Elihu H. Estey 2009]. In the present study, total 16 patients were followed up for cytogenetic studies. Out of 16 patients, 4 patients were with t(8;21) and 12 patients were with t(15;17). From 4 patients with t(8;21), only 1 patient with t(8;21) showed complete cytogenetic response and 1 patient developed clonal evolution. 2 patients showed persistent leukemic activity. Whereas, from 12 patients with t(15;17)
patients, treated with effective modern treatment i.e. a simultaneous All-Trans Retinoic acid (ATRA) plus chemotherapy combination. ATRA combined with CHT results in a significant improvement of remission, disease-free survival and cure rates, particularly when ATRA is simultaneously administered with chemotherapy [Geok YL et al., 2005]. Only 6 patients showed complete cytogenetic response. Though patients treated with ATRA, some patients did not show complete hematologic or cytogenetic response; that was observed in 6 patients. 3 patients did not show response as persistent cytogenetic abnormality was present. Disease progression was observed in 3 patients with clonal evolution in addition to t(15;17). The primary cause of treatment failure with 3+7 therapy is resistant AML in most patients (failure to achieve CR or AML relapse).

In Summary, cytogenetic analysis performed at diagnosis is generally recognized as the single most valuable prognostic factor in AML. Characterization of patients with AML according to presentation karyotype provides an important basis for selection of therapy. These chromosomal abnormalities are important for prognostic purposes and have been equally important for cloning genes involved in leukemogenesis. FISH helps in identification of cryptic abnormalities that are not evident in banding studies. With help of FISH techniques, different new variant partners can be identified. M-FISH is a valuable FISH based cytogenetic tool to refine complex karyotypic aberrations and identify the chromosomal origins of marker chromosomes in hematologic malignancies. Identification and documentation of novel, rare and recurrent chromosomal abnormalities and their frequency make it possible to identify specificity of chromosome abnormality and disease subtype. Together, these observations support the notion that AML is heterogeneous at the molecular level, and in addition its role in diagnosis, cytogenetics is an independent prognostic factor in AML, providing the framework for a stratified treatment approach for this disease.