Prologue
Advancements in Cytogenetics: The advancement of cytogenetic techniques after 1950s, made possible to correlate specific chromosomal abnormalities with various diseases. It quickly documented the central role of cytogenetics in medicine. It was established that cytogeneticists can extract far more information about the human genome than just the chromosome number. Each chromosome could be easily recognized even in the highly rearranged karyotype of tumour cells. With the advent of chromosome banding methods, classical staining methods were employed in the clinical analysis of human chromosomes. The chief applications of classical staining were initially in the study of genotoxicity [Barbara JT et al., 2002]. Early karyotype allowed the discovery that some human disorders result from changes in chromosome number or appearance. In 1959, trisomy 21 was shown to be the cause of Down syndrome and abnormalities in the number of sex chromosomes were shown to cause Turner syndrome (X0) and Klinefelter syndrome (XXY), two frequent disorders of sex differentiation. Today, the resolution and sensitivity of analysis have improved more than 10,000 fold in a very short time. t(8;21), the first recurrent translocations in leukemia was described by Rowley and this basic discoveries led to the targeted drug therapy [Druker BJ 2006].

Cytogenetics in Cancer: Cytogenetic abnormalities are a characteristic attribute of cancer cells. To date, clonal chromosomal aberrations have been found in all major tumor types from more than 58,000 all cancer patients [Mitelman 2010] (Accessed on August 15). Further, their identification continues as a result of technical improvements in conventional and molecular cytogenetics. The World Health Organization (WHO) classification of tumours recognizes a growing number of such genetic changes and uses them to define specific disease entities. Many of these aberrations have emerged as prognostic and predictive markers in hematologic cancers and several types of solid tumors. Furthermore, the molecular characterization of cytogenetic abnormalities has provided insights into the mechanisms of tumorigenesis and has, in a many instances, led to treatment that targets a specific genetic abnormality [Fröhling S 2008].
Boveri's theory on clonal origin in cancer was proved in 1960 when Newell and Hungerford [1960] recognized the Philadelphia chromosome (Ph) in patients with chronic myelogenous leukemia (CML). The issue of chromosomal translocation as a common trait in various forms of cancer became significant with diagnostic and therapeutic value [Druker BJ 2006]. Translocations of chromosomes are a very common event in cancer. Some products of the translocation may lead to develop targeted drugs as in CML and gastrointestinal stromal tumors (GIST). In reviewing recurrent chromosomal translocations in human cancer, Mitelman [2000] recorded over 600 acquired, recurrent, and balanced translocations. In hematologic disorders, the translocations are usually balanced. It may even be that, translocations are secondary or perhaps even tertiary event in cancer. The first step necessary in translocation could be a mechanism that allows the affected chromosomes to stick together. The selection of the segment of chromosome to be translocated is shrouded in total mystery [Koss LG et al., 2007].

The importance of translocations in the classification of the leukemias has been highlighted recently by WHO and various leukaemia patients are categorized solely by cytogenetics [Grimwade D et al., 2009]. Cytogenetic characterization has therefore replaced morphological analysis in the classification of several leukaemias. Translocation type is also crucial in determining the most appropriate therapy and can be used to monitor the therapeutic response in leukemia patients. For example, an Acute Promyelocytic Leukaemia (APML) patient that carries the t(15;17) is likely to respond to therapy with all-trans retinoic acid.

**Human cytogenetic from microscope to microarray:** After identification of translocation next step is to identify genes present in these breakpoints of chromosomal rearrangements. This step can be accomplished by using techniques that physically separate abnormal and normal chromosomes. In 1986 Pinkel et al., [1986] developed a method to visualise chromosomes using fluorescent-labelled probes called fluorescent in situ hybridisation (FISH). These advances in understanding of the genetic basis of myeloid malignancies provided important
insights into disease pathophysiology and novel therapeutic approaches [Frohling S 2005]. FISH helps researchers to zoom in on the defect from the cytogenetic to the molecular level and importantly, they have yielded rough maps for navigating the genome and for allowing more detailed molecular mapping and sequencing. These advances have led to the establishment of chromosome patterns as diagnostic and prognostic indexes in an array of acute and chronic leukemias and lymphomas. It could reveal key information in bone marrow transplantation and to guide for the localization of oncogenes and tumor suppressor genes that are apparently responsible for the development of neoplastic states. The information enables the clinicians to plan the therapy, appraise diagnosis and plan follow-up. FISH paved the way for a more powerful technology called spectral karyotyping (SKY) or multicolour FISH (M-FISH). M-FISH allows all of the 24 human chromosomes to be painted in different colours in a single assay. The main applications for M-FISH have been in leukemias, which are often characterised by complex karyotype in AML and Acute lymphocytic leukemia (ALL). With the introduction of M-FISH techniques in 1996 the comprehensive analysis of complex karyotype and the identification of new cryptic translocations and ultimately the identification of new disease subgroups were possible. Till 2003, more than 600 cases of AML and MDS were analyzed with these molecular cytogenetic techniques [Tchinda J et al., 2003]. The advent of molecular cytogenetic techniques, such as FISH, M-FISH and array-based comparative genomic hybridization (CGH), has added a further level of sophistication to the analysis by which chromosomal breakpoints involved in structural rearrangements now can be mapped very precisely, even within genes. To date, many genes have been identified with advance cytogenetic approaches. These genes are found to be involved in benign and malignant neoplastic disorders. Importantly, these genes represent a substantial proportion of all mutated genes that have been implicated in oncogenesis.

The current scenario of clinical cytogenetics: Despite the fact that cytogeneticists are developing molecular approaches for deciphering the structure, function and evolution of chromosomes; conventional cytogenetics using regular
banded chromosomal analysis remains a simple and popular technique to get an overview of the human genome. Routinely banded karyotype analysis can now be combined with M-FISH and various other molecular techniques, leading to more precise detection of various syndromes. The combination of CGH with M-FISH was seen from the beginning to be a powerful combination for characterising complex karyotypes. More recently, microarray-based formats using large insert genomic clones, cDNAs or oligonucleotides have replaced metaphase chromosomes as DNA targets, providing higher resolution and the ability to directly map the copy number changes to the genome sequence. In other words, chromosomal abnormalities exist as nature's guide to the molecular basis of many unexplained human disorders. Thus, advancement in cytogenetics studies is bound to continue to be indispensable tool for diagnosis, treatment planning and management of genetic disorder [Thirumulu PK and Alwi ZB 2009].

In majority of the patients with AML, acquired clonal chromosome aberrations and numerous recurrent karyotype abnormalities have been discovered. These findings at the chromosomal level could enable molecular studies to identify genes involved in the process of leukemogenesis. The identification of specific chromosomal abnormalities and their correlation with cytomorphologic features, immunophenotype and clinical outcome have led to the understanding of AML as a heterogeneous group of distinct biologic entities. Cytogenetic abnormalities in AML are used as a major criterion for classification and for the understanding of pathogenetic mechanisms in the clinical context. It is also useful to gain a better understanding of the full genetic and molecular events of chromosome rearrangements, as this is the best route for developing cancer-specific designer drugs [Schoch C. 2002].

**Role of cytogenetics in Acute myeloid leukemia:** AML is very heterogeneous at the cytogenetic and molecular genetic levels. Over the last 30 years, several specific recurrent chromosome aberrations have been described in AML [Mróz K 2008]. Acquired cytogenetic aberrations are detected in 55–75% of newly diagnosed patients with AML; the rest show no cytogenetic changes, and this masks any clues
to their molecular pathogenesis. Individuals with normal cytogenetics constitute a large group of AML patients. Many karyotypic abnormalities are associated with specific disease subtypes, characteristic morphologic and immunologic profiles, and distinct therapeutic and prognostic implications [Scoch C et al., 2004]. They are mainly observed as unbalanced and balanced rearrangements and are present as reciprocal translocations, insertions, and inversions. Balanced chromosomal rearrangements are detected in approximately 25–30% of adults with de novo AML. These rearrangements have attracted a great deal of attention. The molecular dissection of these rearrangements has led to identification of several genes that are involved in leukemogenesis mainly due to specific translocations and inversions associated with clinical features and treatment outcome of patients [Mrózek K 2008].

The current trend in clinics is to immediately carry out karyotyping on the cells of a leukaemia patient before treatment, as the identification of chromosome aberrations remains the best known way to predict how a patient will progress or respond to treatment [Sahin FI et al., 2007]. Based on cytogenetic abnormalities, AML is divided into 3 different risk groups which are currently in clinical use; (i) Favorable risk group, (ii) Intermediate risk group and (iii) Adverse risk group. Several translocations; such as t(8;21), t(15;17) and inv(16) are associated with a positive response to treatment and long term survival in AML. On the contrary, a few translocations such as those involving the \textit{MLL} gene on chromosome band 11q23 are associated with poor prognosis. Patients with complex karyotype in adverse risk group also predict poor prognosis [Frohling S et al., 2006, Mrózek K 2006].

\textbf{Aims and Objectives:}

In approximately 60% of patients with AML, pre-treatment cytogenetic analysis reveals an abnormal karyotype. 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 2004]. Cytogenetic aberrations detected at the time of AML diagnosis constitute the
most common basis for predicting clinical outcome in terms of minimal residual disease [Mrozek K et al., 2004]. Molecular genetic alterations are increasingly being used to further refine the prognosis. The reports from India to document cytogenetic abnormalities in AML patients are lacking. Especially, a study of cytogenetic in a large series of AML is not reported. Therefore, to evaluate the clinical significance of cytogenetics in AML, the present study was carried out with following major AIMS:

> Identification of loss and gain of chromosome numbers and its frequency and its impact on prognosis of the patients in AML.
> Identification of rare, novel and recurrent anomalies in different subtypes of AML.
> Evaluation of significance of chromosomal rearrangement for diagnosis, disease progression and prediction of therapy response; at disease onset and during the course of the disease.

The study was undertaken with following OBJECTIVES to fulfil the aims.

> Karyotyping analysis in AML patients with conventional cytogenetic approach.
> Study of primary chromosomal anomalies and its correlation with secondary cytogenetic changes in AML.
> Identification of cytogenetic risk groups of AML patients.
> Study of significance of cytogenetic abnormalities in cytogenetic risk group.
> Documentation of rare novel and recurrent anomalies along with clinical and laboratory work-up details for delineating a disease sub-group, its epidemiology, and response to therapy to global database.
> To find out the cryptic and variant rearrangements using various FISH probes.
> To identify cryptic chromosomal rearrangements in complex karyotypes with the help of M-FISH.
> To evaluate the occurrence and type of genetic abnormalities by the means of FISH in patients with normal karyotype.
> To set up a fast FISH screening for the most common chromosomal abnormalities in AML.
➢ Study of involvement of numerical chromosomal anomalies and their frequency to establish distinct entity.
➢ Study of involvement of structural abnormalities and their frequency in correlation to disease entity.
➢ To find out non random chromosomal aberrations in addition to common aberrations.