REVIEW OF LITERATURE

Research on "Cytogenetics and Molecular genetics" are widely in clinical use for the diagnosis and critical evaluation of Prognosis in Cancer patients. Various discoveries on Chromosomal abnormalities and oncogene detection are being made since few decades. But, the exact genetic facts for the cause of Cancer is yet to unveil and hence Scientists throughout the world are working for it, and to discover better treatment for the patient's survival.

The first discovery in Cytogenetics was started with the observation of human chromosomes in dividing cells and also the disease leukemia was described (Virchow, 1857). Observation in Stages of Mitosis (Von Torok, 1874), Introduction of terms chromatin and Chromosomes, recognition of Acute and chronic Leukemias as two entities were reported.

After all these basic discoveries, the biology of Cancer and determined chromosomal number in humans were described (Van Hansemann, 1890).

Acute Leukemia was separated into two Categories (AML and ALL). Study on Tumor Viruses began (Ellerman & Bang, 1908), and Virus as a causative agent of cancer reported in Fibrosarcoma (Rous, 1911).

Somatic theory of Mutation was described by Boveri (Boveri, 1914) and reported the presence of X and Y sex Chromosomes. Presence of heterochromatins in the chromatids was described (Emil Heitz 1928).
To obtain fine morphology of chromosomes, hypotonic salt solution and a spindle poison were used (Hsu, 1952 & Hughes, 1952). The molecular structure of DNA was described as "double helical strands" (Watson & Crick, 1953).

Morphology and behaviour of Neoplasms was proposed. The correct human chromosome complement as 46 was reported (Tijo & Levan, 1956). New technique "Air-dry" method for improved chromosomal preparation was demonstrated (Rothfels & Siminovitch, 1958).

The year 1960 was the exiting year for the Geneticists. Various discoveries were made during this year. A simple method for short-term peripheral blood culture was developed and the clear Chromosomal preparations were observed (Moorhead, et al, 1960).

Phytohaemagglutinin (PHA), a mitogen was introduced for the better introduction of mitosis in cultures (Nowell, 1960). These methodological improvements, paved a way for a first spectacular discovery for the diagnosis of human cancer. First chromosomal abnormality, the Philadelphia (Ph') Chromosome was described in Chronic Myelogenous Leukemia (CML) Patients (Nowell & Hungerford, 1960).

Certain Physical, Chemical, and Biological agents causing Chromosomal aberrations in Vitro was discovered (Chu, 1962). Harvey Ras Virus causing tumors in mice was identified (Harvey, 1964).
Cell Sorter instrument was introduced to analyse the cells (Kamentsky, 1965), and cell volume difference was examined using blood lymphocytes, (Van Dilla et al, 1967).

First banding technique for the identification of individual human chromosomes-Q-banding using Quinacrine mustard was introduced (Caspersson et al, 1968). Clinical outcome (Prognostic features) were estimated in CML Patients (Whang-peng, 1968).

Flow Cytometer was introduced to measure the DNA content of a cell (Van Dilla et al, 1969). Oncogene hypothesis of cancer was proposed (Huebner & Todaro, 1969).

Centromeric banding (C-banding) was introduced (Pardue & Gall, 1970). Philadelphia negative (Ph-ve) chromosomes were analysed in CML cases (Ezdinli et al, 1970). Giemsa banding technique (G-banding) was introduced to prepare permanent stained slides. (Sumner et al, 1971). Later on, minor modifications were made in G-banding for rapid use (Seabright, 1971), and its mechanism was described (Comings, 1978).

Constitutive heterochromatic regions were determined using C-banding (Arrighi & Hsu, 1971). A three-letter code was introduced in banding techniques (Paris Conference, 1971). Ba (OH)₂ - Barium hydroxide was substituted for Sodium hydroxide in C-banding (Sumner, 1972).

The reciprocal nature of the translocation between chromosomes 9 and 22, in Philadelphia Chromosome was first described (Rowley, 1973).
DNA was analysed in individual cells using Fluorescence activated cell sorter (FACS) (Panet and Khorana, 1974). First international Symposium was conducted and Chromosomal aberrations associated with different Cellular DNA content was observed (Barlogie et al, 1975). DNA Index study was introduced using Flow Cytometry and described in Leukemias and Lymphomas (Barlogie et al, 1977). DNA aneuploidy study was analysed in NHL cases (Costa et al, 1981).

In the field of Molecular genetics, the separation of DNA into fragments using Gel electrophoresis by Southern blotting method was the first technique used (Southern, 1975). Later on, a breakthrough occurred upon the discovery of Taq DNA polymerase, which was first isolated from Bacterium Thermus aquaticus (Chien et al, 1976). Studies on Tumor Viruses was described (Tooze, 1980). Amplification of genes observed as double minutes (dmin) and Homogeneously staining regions (hSR) were described (Schimke et al, 1980).

Chromosomal breakpoints associated with Oncogene activation was proposed (Klein, 1981) and this association was extensively studied (Rowley, 1984). Specific Chromosomal aberrations were highly predicted with survival period of ALL patients (Secker - Walker, 1982). Studies on molecular basis of normal cellular genes (Proto-oncogenes) were made (Varmus, 1984).

Polymerase chain reaction (PCR) was first described and introduced for in vitro amplification of DNA sequences (Saiki et al, 1985). Specific rearranged oncogenes were detected by utilising PCR and various leukemias were diagnosed and prognostic values were evaluated (I, II and III MIC

Specific Oligonucleotide primers have been used for the detection of rearranged oncogenes in Leukemias. (Eisenstein, 1990). To date, 230 primary neoplasia associated Chromosomal aberrations, and over 60 specific rearranged oncogene(s) associated at its specific breakpoints have been detected (Rabitts, 1994 and Heim & Mitelman, 1995).

**Chronic Myelogenous Leukemia (CML)**

CML was first described and its classic features of blood purulence and marked splenomegaly was noted (Virchow, 1845, Bennett, 1845). The first Cardinal feature, Philadelphia Chromosome in CML was described (Nowell & Hungerford, 1960). Philadelphia (Ph\(^1\)) Chromosome was reported in 85% of the CML cases studied (Whang - Peng et al, 1968). Occurrence of Philadelphia Positive and Negative CML cases were described (EZdinli et al, 1970). Ph Chromosome as a balanced translocation (ie) t(9;22)(q34;q11) was well documented (Rowley, 1973), and represented as a single strongest evidence for the diagnosis of CML (Nowell, 1974).

A conflicting clinical correlations were reported in CML. Formation of newer chromosomal aberrations reported to have different survival periods (Sonta & Sandberg, 1978). CML cases with only Ph chromosomes reported to have a higher remission and a longer survival period (Prigogina et al, 1978).
No loss of DNA in t(9;22) and acquisition of additional abnormalities during blast crisis phase was observed (Rowley, 1980). These findings were further scrutinized in CML cases (Hagemeijer, et al, 1980). Various Cytogenetic abnormalities were described in correlation with its types, and clinical features (Third International workshop, 1981).

C-ABL oncogene mapped to Chromosome 9q34, BCR oncogene mapped to chromosome 22q11 was reported to have rearranged in CML cases (de klein et al, 1982). Chronic and acute phases of CML, its Karyotypic progression during acute phase with +8, i(17q), +19 and extra ph1, and also Hyperdiploidy was extensively described (Rowley, 1983).

Function of C-ABL and BCR, its tyrosine kinase activity were reported (Davis et al, 1985). Blast crisis karyotypic evolution was correlated with poor prognosis and survival (Alimena et al, 1987). The key role in the transformation of myeloid cells, resulted in large ABL fusion product with 210 KD and 8.5 Kb mRNA transcript having increased enzyme activity were extensively observed (Westbrook, 1988). These features were further evidenced (Daley et al, 1990)

Recurring Chromosomal aberrations were often correlated with clinical features and patient’s response to therapy (Trent et al, 1989). 50 CML cases were reviewed for Ph negative observed during excess blasts (Travis et al, 1986).
Intensive Chemotherapy, IFN α and allogeneic Bone marrow transplantation (BMT) has shown to have great potential in inducing complete remission (Allan et al, 1995)

Fluorescent In Situ Hybridization (FISH) techniques using non-radioactive probes were introduced and established. Detection of Ph chromosome using FISH has become routine in Cytogenetic labs (Cremer et al, 1986 and Smit et al, 1990).

Use of PCR and RT-PCR for the detection and amplification of BCR-ABL oncogene rearrangement has revolutionized as a most sensitive and rapid method (Cross et al, 1994; Junia et al, 1996 and Thomas et al, 1996).

**Chronic Lymphocytic Leukemia (CLL)**

Natural history was first described (Minot and Isaacs, 1924) and clinical staging was proposed for precise diagnosis (Binet et al, 1977). The monoclonal nature, specific chromosomal aberrations and oncogene rearrangements were well described (Silber et al, 1990).

Specific mitogens were used to increase the mitosis to estimate prognostic signs (Ross et al, 1987). Trisomy 12 was reported as a frequent cytogenetic abnormality and analysis of other abnormalities were increased with banding methods (Han et al, 1987). 95% of B-Cell CLL and only 5% of T-Cell CLL were reported (Bird et al, 1989).
Chromosome 14 abnormality as del 14q, inv (14q) and t(11;14)(q13;q32) were observed as second most common karyotype abnormalities which revealed poor prognosis. Reports revealed location of Ig H gene on 14q32 and TCR alpha on 14q11 (Kipps, 1993 and McManus et al, 1994).

Clonal chromosomal abnormalities of +12, 14q+,13q- were reported in various studies conducted on thousands of CLL cases. All these revealed poor prognosis and survival period. Only Normal diploid karyotypes revealed good prognosis. (Juliusson et al, 1993; Caguioa et al, 1994 and Matutes et al, 1996).

FISH technique have been widely used for the detection of +12, 13q14 (Witzig et al, 1994 and Mould et al, 1996). High incidence of -18 was also described (Younes et al, 1994).

Oncogene rearrangements - Bcl 1 on 11q13 in t(11;14)(q13;q32); Bcl 2 on 18q21 in t(14;18)(q32;q21), and Bcl 3 on 19q13 in t(14;19)(q32;q13) were elaborately described (Raghoebier et al, 1991 and Dyer et al, 1994).

Oncogene MDM2 seemed to have rearranged in +12. (Mats et al, 1996). RT-PCR use was described in various CLL cases (Jan et al, 1996). Cytotoxic agent Fludarabin was reported to have increased high remission in CLL (O'Brien et al, 1995).

Acute Lymphoblastic leukemia (ALL)

The cytogenetic aberrations of ALL were observed, and correlated with survival period (Zeulzer et al, 1976, and Raimondi, 1993). The first description
on predominant occurrence of hyperdiploids in Childhood ALL, showed as a significant feature. (Oshimura, 1977). Specific Karyotypes were analysed for its survival period.

Hyperdiploidy was observed to have better prognosis (Williams, 1982), and Hypodiploidy had poor prognosis (Pui, et al, 1987). High-risk and low-risk ALL cases were analysed (Bloomfield et al, 1986).

DNA aneuploidy study using Flow cytometric study analysis was described. Cellular DNA content was correlated with the numerical chromosomal abnormalities (Kamihira et al, 1994).

Presence of Philadelphia chromosome in ALL was observed (Bloomfield et al, 1978). 25% of adults, and 2.5% of children were reported to have Philadelphia chromosome (Pui, et al, 1990). BCR-ABL oncogene was rearranged with 185 to 190 KD protein and 6.5-7.4 kb fusion mRNA transcript was described and detected using RT-PCR and PCR analysis using specific primers (Glassman, 1995). FISH technique was used to detect BCR-ABL rearrangement in Ph+ negative chromosome ALL cases (Shah, 1993, Preti et al, 1994). Presence of Ph chromosome in adults was described to have poor prognosis than in children (Maung et al, 1995).

Pre-B Cell ALL with L1 morphology associated with t(1;19)(q23;p13) was described and early failure in treatment was experienced (Carroll et al, 1989). Children with t(1;19) observed to have poor prognostic outcome (Brisco et al, 1994). the molecular basis of t(1;19) was studied, and a new homeobox fusion mRNA of E2A and PBX 1 was described (Hunger et al, 1991, and Troussard
et al, 1995). RT-PCR was used to detect t(1;19) & its rearranged oncogenes. This was also widely described during follow-up and Minimal residual disease detection. (Privitera et al, 1994 and Enrica et al, 1996).

t(4;11)(q21;q23) in ALL L1 or L2 morphology was first described in children and later confirmed by various investigators, which conferred poor prognosis (Stong et al, 1985). Even infants less than one year of age was described (Cimino et al, 1993). MLL/HRX gene was rearranged, located at 11q23. This was reported using RT-PCR analysis, and observed to have poor prognosis and response to therapy. Monosomies of 5 and 7 and also 5q-, 7q- were described in developed secondary leukemias (Joanne et al, 1995 and Yanming et al, 1996).

T-cell ALL was relatively few and fall in L3 type. t(8;14)(q24;q11) was first described with male predominance (Trent et al, 1989). The sporadic occurrence of other translocations were, t(11;14)(p13;q11), t(10;14)(q24;q11) and t(1;14)(p34;q11) and also 12p12 (Martin et al, 1995 and Ramana et al, 1995). (IgH) Immunoglobulin heavy chain gene located on 14q32 was described (Fishel et al, 1990). T-cell receptor (TCR) genes were reported to have located on chromosome 7 (Trent et al, 1989). TCR α in 14q11, TCR β in 7q32 and TCR γ in 7p13 were reported (Carr et al, 1995).

FISH was used to detect IgH and TCR gene rearrangements (Martin & Lawlor, 1991). RT-PCR and PCR analyses were in extensive use and still continuing (Kang et al, 1995). Recently patients prognosis and survival rate were increased with newer treatment modalities (Evensen et al, 1994).
Acute Non-Lymphocytic Leukemia (ANLL)

Myeloid lineage types of M₁-M₇ was proposed by FAB group. (Bennett et al., 1985; Koeffler, 1987). Large number of patients were studied. Every subtype having specific chromosomal aberrations and rearranged oncogenes localized at its specific breakpoints were described (Mitelman, 1988).

Every patient of ANLL was reported to have abnormal Karyotype (Yunis et al., 1981). In t-ANLL (therapy related), +8,-7 & 7q⁻ were reported frequently (Samuels et al., 1988). Predominance in elders (>60 yrs) was reported (Cartwright & Staines 1992). Diagnosis, Prognosis and response to therapy were well documented (Fourth International workshop, 1984).

Acute myeloid leukemia (AML) M₁ and M₂ types were reported to have 2-3% of Ph chromosome, with P210 KD protein BCR-ABL oncogene which indicated poor prognosis (Borkhart et al., 1993 & Juan et al., 1995). Recurrent Chromosomal abnormalities reported were 11q23 in t(11;15)(q23;q14) and +5 with short survivals (Hernandez et al., 1995, Rios et al., 1995).

Acute promyelocytic leukemia (APL) M₃ type was reported with t(15;17)(q22;q12), observed in majority of younger adults (Mitelman, 1991). PML/RAR-α (Promyelocytic leukemia/retinoic acid receptor alpha) chimeric gene was described to have rearranged. Studies using RT-PCR implied high response rate with gond prognostic values (Viniou et al., 1995).

Acute myeloblastic leukemia (AML): M₂ type was described with t(8;21)(q22;q22), observed in adults and children. This translocation revealed favourable prognosis in most of the reported cases (Kita et al., 1994). AML-ETO oncogene was described as a fusion mRNA transcript using RT-PCR (Valirie et al., 1995).
t(6;9)(q23;q34) with rearranged Pim-1 oncogene on 6q23 was reported (Von Lindern *et al.*, 1990) in AMML. AMMO-L-M₄ Eₒ (ie) with eosinophils was reported to have inv (16) (p13q22), associated with good prognosis (Liu *et al.*, 1993a). 5¹-CBF/MYH 11-3¹ (core binding factor β gene/Myosine heavy chain) fusion gene was reported in inv(16) by FISH and RT-PCR (Paula *et al.*, 1995).

Acute monoblastic leukemia (AMOL) M₅ type was reported with Cytogenetic abnormalities of 11q23. This was seen in children expressing MLL Gene. (Bernard & Berger, 1995) 11q23 was reported to have translocated with other chromosomes of 1,9,6 and 22 (Osamu *et al.*, 1996).

Acute Erythroleukemia (AEL) M₆ type was reported to have del (20)(q11) in very few cases. (Richard *et al.*, 1995).

Acute Megakaryoblastic leukemia (AMKL) M₇ type was described recently. Patients were younger than other subtypes and had poor response to therapy. +21 was reported in few cases (Ribeiro *et al.*, 1993). +8, -7 were reported using Interphase cytogenetic study (Zipursky *et al.*, 1994a).

t(1;22)(p13;q13) and t(7;11) (q11;q24) were reported (Lu *et al.*, 1993) in AMKL.

Ig and TCR α,β,γ were described to have rearranged in most of the ANLL cases (Adriaasen *et al.*, 1991). +4 and dmᵦ (double minutes) also were described in t-ANLL with poor prognosis (Ramond *et al.*, 1995).

t-ANLL was reported to have 11q23 abnormality, C-myc amplification, dmᵦ 5q- and 7q-, with worst prognosis and survival (Roulston *et al.*, 1995, Elvira *et al.*, 1996).