"Leukemia" - Cancer of haematopoietic origin characterized by acquired genetic rearrangements at presentation and also during clinical course of treatment. Cytogenetics and molecular genetic approaches were preferred to detect specific and secondary chromosomal aberrations for the precise diagnosis of the patients.

Bone marrow aspirates (BMAS), and peripheral blood (PB) samples were collected from 863 clinically diagnosed leukemia patients, coming to Cancer Institute, Madras for Diagnosis and treatment. These patients were comprised of 366 CML cases (42.41%), 364 ALL cases (42.18%), 82 AML cases (9.50%), and 51 CLL cases (5.91%).

Cytogenetically with improved in-vitro tissue cultures and banding (GTG, CBG, and QFQ) methods, specific, secondary, and additional chromosomal aberrations were detected in leukemia patients and analysed by ISCN classification (An International system for Human Cytogenetic Nomenclature 1985).

Flow cytometric analysis by "Flow activated cell sorter (FACS)" using single cell suspensions were used to detect aneuploid clones in leukemia cases. This was analysed in the histograms. In depth study on detection of oncogene(s) and its activation process through altered protein products was performed by molecular genetic approaches using the sensitive and rapid methods "Polymerase chain reaction (PCR)" and "Reverse transcriptase -PCR (RT-PCR)".
In vitro amplification of DNA or RNA (cDNA) samples from leukemia patients were performed in an automated thermal cycler using specific oligonucleotide primers under critical temperatures. The amplified PCR and/or RT-PCR products were analysed by Agarose gel electrophoresis method. The electrophoresed leukemia sample revealed altered protein products at appropriate base pair length (Positive bands) compared with that of DNA marker. BCR-ABL oncogene rearrangement in CML and ALL cases; Immunoglobulin heavy chain (IgH) gene and T-cell receptor gamma (TCR-γ) gene(s) rearrangement in ALL cases were analysed in few leukemia patients.

In 366 Chronic Myelogenous Leukemia patients (CML), Philadelphia chromosome (Ph) (ie t(9;22)(q34;q11) was detected in 91% of cases. Chronic phase (benign) of CML observed with Ph chromosome and normal diploid clones had the best prognosis and highest survival rate (>6 yrs) and response to therapy. BCR-ABL oncogene was detected to have rearranged in 85% of CML cases, analysed by RT-PCR method. The rearranged BCR-ABL fusion protein was detected at 210bp. Acute and blast crisis (malignant) phases were detected with specific, secondary (trisomy 8, monosomy 7, and monosomy 3), and additional (dicentrics, and Ring chromosomes) chromosomal abnormalities observed to have intermediate to poor prognosis which indicated progression of the disease. 10% of CML cases died during acute or blast crisis phases detected having aneuploid clones with multiple complex aberrations. Normal diploids were completely absent. Good response to treatment, highest rate of survival (>6 yrs) were observed in 23% of cases observed with only normal diploid clones.
In 364 Acute Lymphoblastic Leukemia (ALL) patients, 29% of ALL-L₁ type were detected cytogenetically with Philadelphia chromosome. 6% of children and 21% of adult ALL cases were observed with specific Ph translocation reported to have poor prognosis. RT-PCR study of 2 cases revealed BCR-ABL oncogene rearrangement in one case revealed BCR-ABL fusion protein product at 190bp. ALL-L₂ type was detected with t(4;11)(q21;q23) in 16% of cases having poor prognosis. ALL-L₃ type was detected with t(8;14)(q24;q32) specific translocation in 11% of cases, which had poor prognosis. ALL-L₁₁, L₂ & L₃ types were observed to have normal diploids, hyperdiploids, hypodiploids and pseudodiploids. The only significant prognostic factor in ALL was detected in cases having hyperdiploid clones (>55 chromosomes) in childhood ALL. 7 cases of aneuploidy detection using flow cytometry had normal diploids, in 2 cases, hyperdiploids in 3 cases, hypodiploid in 1 case and haploid in 1 case, all revealed poor prognosis and response to therapy.

Secondary chromosomal abnormalities detected were t(1;19)(q23;p13) in 3% of cases indicated poor prognosis in ALL-L₁; monosomy 20, 5q⁻, 7q⁻, trisomy 5; additional chromosomal abnormalities observed were dicentrics, double minutes and ring chromosomes in majority of high-risk group ALL, which revealed poor prognosis and shorter survival period.

60% of ALL cases were detected to have IgH (Immunoglobulin heavy chain) gene rearrangement by PCR method. These cases revealed 90-130 bp length protein product. 57% of ALL cases had TCR-γ (T-cell receptor gamma)
oncogene rearrangement detected at 200 bp length protein product. These cases indicated the clonal evolution of ALL disease.

13% of death was reported in high-risk group ALL with complete absence of normal diploid clones. These cases were detected to have Ph chromosome, t(4;11), t(8;14), dicentrics and double minutes during clinical course. Good prognosis, higher rate of survival (>6 yrs), and good response to treatment were detected in 18% of cases having only normaly diploid clones and/or hyperdiploid clones.

In 82 Acute Nonlymphocytic Leukemia (ANLL) patients, 6% of cases were detected in AML, M₁ and M₂ types with Philadelphia chromosome as specific chromosomal translocation observed with poor prognosis and shorter survival period. 32% of AML M₃ type was detected with t(15;17)(q22;q12) specific chromosomal aberration having good prognosis and survival period. 42% of AML M₄ type had specific chromosomal translocation t(8;21)(q22;q22) observed to have favourable prognosis and higher survival rates. AML-M₅ had the specific abnormality, del(11)(q23) in 10% cases with intermediate prognosis.

Therapy related ANLL cases were detected with secondary chromosomal abnormalities such as t(6;9) in AML-M₄; trisomy 8, trisomy 5, 7q⁻, trisomy 4 and trisomy 21 in majority of cases. Additional chromosomal abnormalities detected were dicentrics, double minutes, and ring chromosomes with hyperdiploid, hypodiploid, and pseudodiploid clones. These abnormalities indicated aggressive progression of the disease associated with occupation or previous treatment of the patient.
8% of cases detected to have died within an year of survival with 90% of aneuploid clones. Complete absence of normal diploid clones were detected in all 8% of dead cases. Good prognosis and response to treatment with higher rate of survival were detected in 18% of AML cases. These cases revealed only normal diploid clones and/or trisomies.

In 51 Chronic Lymphoblastic Leukemia (CLL) cases, the higher incidence was observed in >35-80 yrs of age group. CLL was not observed in children and younger adults (1-30 yrs). Trisomy 12 was the only specific chromosomal abnormality detected in 32.5% of CLL cases. These cases revealed poor prognosis and short survivals.

Secondary chromosomal abnormalities detected were dup(14)(q32) in 15% of cases, del(13)(q14) in 7% of cases, and monosomy 18 in 10% of CLL cases. These abnormality were observed in hyperdiploid and pseudodiploid clones. These cases were observed to have intermediate to poor prognosis, and short survival period due to the secondary event in the pathogenesis of CLL disease.

Normal diploid clones were the only significant prognostic factor detected in CLL cases having best prognosis and higher survival period (>5 yrs) in 10% of CLL cases. 8% of death was reported in CLL cases within 6 months to 2 yrs survival, detected to have +12, 14q+ and few dicentric chromosomes.
To conclude

In CML

i) Ph' chromosome (i.e) t(9;22)(q34;q11) was detected as a specific chromosomal aberration revealed good prognosis with normal diploids.

ii) Secondary chromosomal abnormalities such as trisomy 8, monosomy 3, loss of Y chromosome; and additional chromosomal abnormalities such as dicentrics, double minutes, and Ring chromosomes during acute and blast crisis phases revealed progression of the disease and poor prognosis.

iii) BCR-ABL oncogene was detected in 85% of the cases and observed to have rearranged in CML cases at 210bp length which were mapped at specific chromosomal breakpoints (ABL in 9q34; BCR in 22q11).

In ALL :

i) Specific chromosomal abnormalities detected were
   t(9;22)(q34;q11) in ALL-L₁
   t(4;11)(q21;q23) in ALL-L₂
   t(8;14)(q24;q32) in ALL-L₃

   All these specific abnormalities observed to have poor prognosis and short survival periods.

ii) Secondary chromosomal abnormalities such as t(1;19)(q23;p13), del(1)(q23), del(7)(q22),-20 and additional chromosomal abnormalities such as dicentrics, rings and double minutes observed in high-risk group ALL cases revealed poor prognosis and poor survivals.
iii) Flow cytometric analysis revealed aneuploid clones (hyper, hypo and haploid) in ALL cases. These cases cytogenetically had hyper, hypo and normal diploid clones. This correlative study helped in precise diagnosis of ALL.

iv) Presence of hyperdiploid clones in childhood ALL revealed good prognosis and response to therapy with higher rate of survival period.

v) Specific rearranged oncogene(s) detected were
(a) Rearranged BCR-ABL oncogene at 190bp
(b) Rearranged IgH oncogene altered at 90-130bp observed to have mapped to specific chromosomal breakpoint 14q32.
(c) Rearranged TCR-γ oncogene altered at 200 bp length observed to have mapped to specific chromosomal breakpoint 7p13.

In AML

i) Specific chromosomal abnormalities detected were
   t(9;22)(q34;q11) in AML-M₁ & M₂
   t(15;17)(q22;q12) in AML-M₃
   t(8;21)(q22;q22) in AML-M₄
   del (11q23) in AML-M₅

   t(15;17) and t(8;21) chromosomal abnormalities revealed favourable prognosis and survival periods.

ii) Secondary chromosomal abnormalities such as t(6;9), +8, -5, del (7q22), +21; and additional chromosomal abnormalities such as
dicentrics, double minutes, and ring chromosomes revealed progression of the disease in therapy related ANLL after chemotherapy.

iii) Only normal diploid and/or trisomies revealed intermediate to good prognosis and response to therapy.

In CLL

i) Trisomy 12 was detected as the specific chromosomal abnormality, revealed poor prognosis and short survivals.

ii) Secondary chromosomal abnormalities such as dup(14q32) and -18 were detected during aggressive state of the disease, revealed poor prognosis.

iii) Only normal diploid clones revealed good prognosis, response to therapy and survival period.

Detection of specific chromosomal aberrations were observed to have associated with oncogene(s) rearrangements which were mapped to specific chromosomal breakpoints. Present findings from hundreds of leukemia cases revealed its diagnostic significance in patients and also it greatly helped in evaluating the prognosis and survival of the patient. The correlative study of cytogenetics, flow cytometry and molecular genetics greatly increased the precise diagnosis of the leukemia patient.