DISCUSSION

CHRONIC MYELOGENOUS LEUKEMIA (CML)

Present observation of 366 CML cases revealed incidence of CML in all age groups from children (<10 yrs) to older patients (75 yrs). Observations revealed more common in middle-aged (30-50 yrs) with 227 cases (62%) of male predominance over 139 cases (38%) of females. Higher incidence of CML in late middle aged (40-50 yrs) with slight marked male preponderance was reported by Hughes and Goldman, 1991.

CML cases revealed the most specific and significant chromosomal abnormality, the Philadelphia chromosome (Ph') in 293 cases (91%), observed as t(9;22)(q34;q11). This was reported as a minute chromosome by Nowell and Hungerford, 1960 and represented as a balanced translocation by Rowley, 1973. 85% of CML cases were reported as Ph chromosome positive by Whang-peng et al. 1968 and 90% of Ph chromosome by Groffen, 1984 who reported the monoclonal nature of the disease, and recently reported 90-95% of CML cases with occurrence of consistent Ph chromosome abnormality by Allen et al., 1992. These findings were in accordance with present study, and accepted as a best chromosomal marker for the diagnosis of CML patients.

28 cases (9%) of CML revealed Ph' negative chromosomes in this present study. 9-10% of Ph' negative CML cases were reported earlier (Morris & Fitzgerald, 1992). Cytogenetically this was reported to have masked due to formation of complex translocations.
Evolution of CML through disease transition from benign chronic phase to accelerated acute and aggressive blast crisis phases were noticed cytogenetically with identifiable secondary and additional chromosomal aberrations during progression of the disease. Prognosis of CML patients during clinical course of treatment was described by Sokal, 1987, and reported poor understanding of the mechanism of disease progression.

Present study revealed incidence of trisomy 8 in 91 cases (28%), monosomy 7 in 63 cases (19%), -3 in 37 cases (11%), and trisomy 19 in 19 cases (5%) at higher rate than incidence of extra Ph chromosome in 6 cases (1%) and -Y in 2 cases (1%). Majority of these secondary chromosomal abnormalities occurred during acute and blastic phases. Presence of trisomies were more in acute phase, and monosomies during blast crisis. Mitelman, 1991, reported CML cases with trisomy 8, trisomy 19, i(17q) and monosomies during disease progression. It was stated that most deleterious effects were exerted by monosomies than trisomies due to loss of certain genes at lost chromosomal segments.

Blast crisis phase of CML was observed with dicentrics, double minutes and ring chromosomes. Over expression of genes and loss of genes were reported by the presence of dicentrics and double minutes (Mitelman, 1994).

Cytogenetic analysis of benign chronic phase CML was found with normal diploid clones and Ph chromosome. These patients revealed best prognosis and survival period, noticed during follow-up study. Most of these cases attained complete remission with induction therapy. "Allan et al., 1995
reported 30% of chronic phase CML attained complete remission with interferon-α induction therapy, which was in accordance with present data on CML cases.

Acute phase CML showed >70% of aneuploid clones with hyperdiploids and hypodiploids (+8, +19, -7, and Ph chromosomes). Only 10-20% were found with normal diploid clones. Blast crisis phase was found with higher rate (65%) of -3, -7, dicentrics, and double minutes. Rowley, 1983; and Cervantes, et al. 1986 reported the disease progression during blast crisis and explained the deleterious effects of loss of genes.

Patients of this present study revealed different survival period, intermediate to poor prognosis, and response to treatment modalities. Monosomies revealed shorter survival period of 1 to 2 yrs, trisomies with intermediate survivals and response to treatment of 3 to 4 yrs. Normal diploids were found with best prognosis and survival period of >5-6 yrs. Sonta and Sandberg, 1978; Heim and Mitelman, 1995 reported various conflicting clinical correlations and explained secondary chromosomal aberrations associated with shorter survivals. Prigogina et al., 1978 reported a longer survival period with remissions during chronic CML phase.

Present investigations on CML revealed death in 38 cases (10%) of which 26 cases (7%) were males, and 12 cases (3%) were females. Present data described a higher death rate in 30 yrs of age 26 cases (6%) attained death within 2 yrs and only 9 cases (2%) died within 3-4 yrs. None of a child (<10 yrs) was reported dead during follow-up study. Cytogenetic analysis of these
10% dead cases revealed complete absence of normal diploid clones. 2% were in acute phase, and 8% in blast crisis phase. Monosomies of 7, 3, dicentrics and rings were reported in all blast crisis phase. Alimena et al., 1987 reported a correlation of cytogenetics during blast crisis with poor prognosis and survival period.

Good response to therapy with increased (>5 yrs) survival periods were reported in majority of chronic phase CML. 201 cases (55%) were males, and remaining 127 cases (35%) were females. These patients were observed to continue their clinical treatment. All the 10 cases of children were observed to have alive for >5 yrs. Present data showed 123 cases (34%) of middle-aged, 148 cases (40%) of adults, and 47 cases (13%) of elders with higher rate of survivals.

90% of Ph chromosome clones were observed with normal diploids than hyperdiploids. 12% of cases observed to have +8. 23% of cases observed the highest rate of survival (>6 yrs). 90% of cases observed to have normal diploids and Ph chromosomes. Prigogina et al., (1978) revealed normal diploids in CML cases as a best prognostic factor.

Present data on molecular genetic analyses observed BCR-ABL oncogene rearrangement in Ph positive CML cases. RT-PCR sensitively detected 7 cases (85%) of CML having BCR-ABL oncogene rearrangement using specific pairs of CML oligonucleotide primers. No loss of DNA in t(9;22) was reported earlier (Rowley, 1980). De Klein et al., 1982 reported the mapping of C-ABL oncogene to chromosomal breakpoint 9q34, and BCR oncogene to chromosome 22q11.
cases of chronic phase CML and 21 cases of blast crisis CML were reported with BCR-ABL oncogene rearrangement and also the rapid and sensitivity of RT-PCR in oncogene were detected by Morgan et al., 1990.

85% of CML cases were observed with the rearranged BCR-ABL oncogene as a fusion protein of 210 bp length. Daley et al., (1990), reported CML with BCR-ABL transcribed and translated into a 210kd (P210) fusion protein which was considered central to the pathogenesis of CML. Blennerhassett et al., 1988 reported the chimeric protein product of 210 KDa encompassing 5kb on chromosome 22 at M-bcr (Major breakpoint cluster region) encoding an elevated serine/threonine kinase activity within the first exon of CML cases.

**ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)**

Cytogenetic observation on 364 ALL cases indicated ALL incidence in all age groups from pediatric cases (<10 yrs) to older patients (70 yrs). Present data showed predominance in 241 male cases (66%). Higher incidence was observed in both children and younger adults of <20 yrs. (Heim and Mitelman, 1987) reported the incidence peaking at around 3-5 years of age in ALL, and also more common occurrence in young ALL cases. Males were reported to have affected more often than females.

319 cases (88%) of ALL were observed with chromosomal aberrations of both numerical and structural chromosomal abnormalities, and only 45 cases (12%) of the remaining showed insufficient mitosis. An evolving new chromosomal patterns in ALL cases was reported by Williams et al., 1990.
cases (42%) of ALL showed only numerical chromosomal abnormalities and 183 cases (57%) revealed both structural and numerical chromosomal abnormalities in this present investigation.

Present study revealed occurrence of all the three types (ALL-L₁, L₂&L₃) with specific chromosomal translocations emphasizing its importance in diagnosis and evaluation of prognosis. 330 cases were reported with 66% of clonal chromosomal abnormalities including Ph chromosome t(4;11), t(8;14) and 14q⁺. The fourth International Workshop, 1984 also reported its prognostic features of specific translocations.

94 cases (29%) of ALL-L₁ revealed Ph chromosome as specific chromosomal translocation with a male predominance of 65 cases (20%) over female 29 cases (9%). Pediatrics (<10 yrs) 20 cases (6%) were found with Ph positive chromosomes, and 73 cases (21%) of adults were found Ph positive. This present data was compatible with that of Ribeiro et al., 1987, who analysed 366 ALL cases and reported 141 cases (38.5%) with chromosomal translocations. 2-6% of Ph positive chromosomes reported in children, and 17-25% in adult ALL cases.

Ph chromosome was observed with normal diploids and/or hyperdiploid clones. 13% of cases revealed hyperdiploid clones (>50 chromosomes) at diagnosis which showed good prognosis and response to treatment. Pui et al., (1990) reported patients with >50 chromosomes observed in 1/4th of childhood ALL cases and proved to have most durable responses to therapy.
52 cases (16%) of ALL L₁&L₂ were observed with t(4;11)(q21;q23) translocation. 34 cases (11%) were males and only 18 cases (6%) were females. Higher incidence of this specific translocation was reported in 30 cases (9%) of children (<10 yrs) and 15 cases (4%) in younger adults. Strong et al., 1985 reported t(4;11) in a acute leukemic cell line. 11q23 involved in t(4;11) was found predominantly in infant ALL cases in this present study. This interesting view of incidence in ALL cases found in accordance with reports' of Joanne et al., 1995 who explained chromosomal breakpoint 11q23 as an "hotspot" translocation.

Only 37 cases (11%) of ALL-L₃ revealed t(8;14)(q24;q13) abnormality with 24 cases (8%) of male predominance than 13 cases (4%) of females. Third International workshop, 1981 reported 10% of ALL-L₃ with t(8;14) translocation. Adults (9%) were found affected more than children and older patients (2%). t(8;14) abnormality having CNS involvement during patient's clinical course was reported by Trent et al., 1989. Present study revealed 10% of ALL cases present along with aneuploids and other secondary and additional chromosomal abnormalities such as -7, -5, dicentrics and double minutes. An earlier report by Williams et al., 1984 suggested translocations and ploidy increase the proliferative rate of malignant cells, and amplification of genes associated with resistance to therapeutic drugs.

Present study revealed, children (<10 yrs) with marked hyperdiploid clones (>50 chromosomes). >25% of this present study of children showed hyperdiploids with good prognosis and higher rate of survival. Reports on chemotherapy with methotrexate and cytosine arabinoside in children with
hyperdiploids (>50 chromosomes) observed good response to treatment and increased survival period (Kaneko, 1982; GIMENA cooperative group, 1989).

t(1;19)(q23;p13) in ALL-L1 was observed as a secondary chromosomal abnormality in 10 cases (3%) which showed poor prognosis. 5-6% occurrence of t(1;19) with low WBC count was reported previously by Raimondi et al., (1990).

Specific and secondary chromosomal abnormalities were observed in 35% of high-risk group ALL involving majority of monosomies, deletions, and dicentrics. Previous reports examined hypodiploid clones having -20, t(1;19), deletions having poor prognosis (Pui et al., 1987). Present study of monosomy 20 in 8% of 28 cases were in accordance with earlier reports. 15% of high-risk ALL had pseudodiploids, haploids, 5q-, 7q-, dicentrics, double minutes. Pui et al., 1990 reported high-risk group ALL with poor prognosis due to many chromosomal deletions and loss of chromosomes.

50% of cases were observed as low-risk group ALL having only numerical chromosomal abnormalities. Hyperdiploids were most frequently observed with 47-49, and 50-55 chromosomes. +5 found in 6%. 10% of these cases attained complete remission in children. Pui et al., 1990 b reported >50 chromosomes having good prognosis and attainment of complete remission (CR) in low-risk group ALL.

48 cases (13%) of death was reported in present ALL cases, in which 33 cases (9%) of males, and 4% of females were observed. These patients carried specific translocations, hypodiploid clones, and dicentrics. Although an adult male ALL case observed 7 yrs survival, who died after BMT was due to Ph
chromosome positive with pseudodiploid and hypodiploid clones. 3% of cases observed to have Ph chromosome and monosomies; 4% with t(8;14), dicentrics and double minutes survived for <2 yrs. 13% of dead cases interestingly noted to have complete absence of normal diploid clones. 8 yrs survival in adult ALL cases was reported by Evensen, et al., 1994.

Good survival was observed in 316 cases (87%) of this present study, showed intermediate to good prognosis and response to therapy. Higher incidence of survival was observed in 112 cases (31%) of children, and 155 cases (43%) of younger adults. 50% of cases were observed to have low-risk group. Normal diploids and hyperdiploids were observed in >55% of cases. >6 yrs survival was observed in 68 cases (18%). Even 2 months old male kid, and 8 months old female kid were found to have good prognosis and > 6 yrs survival.

7 ALL cases were studied for flow cytometric analysis, which revealed aneuploid (hypodiploid, hyperdiploid, and haploid) and diploid clones in all the cases. These cases cytogenetically showed to have hyperdiploid (>47), hypodiploids (<40-45), and normal diploid clones. This correlative study on both cytogenetics and flow cytometry to increase the diagnostic significance and to evaluate prognosis was highlighted in a report (Kamihira et al., 1994).

Molecular genetic approaches using PCR and RT-PCR methods revealed BCR-ABL oncogene rearrangement in a ALL case, studied out of 2 cases. Cytogenetically this BCR-ABL positive ALL case was found as Ph negative. Westbrook et al., 1992 reported the presence of BCR-ABL positive ALL cases
with P190. This report was in agreement with that of present data having 190bp protein product, BCR-ABL rearrangement in ALL cases were reported to have rearranged within M-bcr (major breakpoint cluster region), but further upstream at m-bcr in the first intron of BCR gene exhibiting elevated tyrosine kinase was reported by Gale and Butturini, 1990.

60% of 6 ALL cases were observed with IgH immunoglobulin heavy chain gene rearrangement in both sexes. PCR method detected 90-130bp length protein product. IgH gene mapped to 14q32 was determined by molecular methods (Grimaldi and Meeker, 1989).

57% of 4 ALL cases were observed with TCR-γ-(T-cell receptor gamma) gene rearrangement at 200 bp length. TCR-γ mapped to 7p13 chromosome was reported by Carr et al., 1995 in most of T-cell ALL having 200 KDa protein.

Ig H and TCR-γ oncogene rearrangements usually observed in lymphoid malignancies was examined by McCarthy et al., 1992. The usefulness of PCR and RT-PCR methods for detecting clonal evolution in ALL cases and also detection of minimal residual disease was highly documented (Potter et al., 1993).

ACUTE NONLYMPHOCYTIC LEUKEMIA (AML)

82 cases of AML revealed higher incidence of 62 cases (75%) in middle-aged, a lower incidence 15 cases (18%) of children and 5 cases (6%) of elders. 56 cases (68%) of males showed predominance than 26 cases (32%) of females. Higher incidence after 55-60 yrs of age and male predominance were reported by Heim and Mitelman, 1987.
Over 75 cases (91%) of AML revealed both numerical and structural chromosomal aberrations. Yunis et al., 1981 reported 96% of AML cases showing karyotypic abnormalities, which was in accordance with present study.

Cytogenetic analysis described in AML M₁ to M₅ types revealed specific chromosomal abnormalities. 5 cases (6%) of Ph chromosome was observed in AML M₁ and M₂ types. Allen et al., 1992 reported a smaller proportion of AML cases can also be Ph positive, and discussed the role of molecular approached for monitoring Ph-positive disease. These patients showed higher incidence of males (5%) than (1%) females, and observed to have poor prognosis and response to treatment. Ph positive AML cases were reported with BCR-ABL rearranged oncogene of P210 protein product with poor prognosis and very short survival periods (Juan et al., 1995).

24 cases (32%) of AML-M₃ in this present investigation revealed t(15;17)(q22;q12) chromosomal abnormality, 16 cases (21%) of males, and 8 cases (11%) of females were affected. Higher incidence of t(15;17) abnormality was observed in 22% of younger adults, and lower incidence of 5% in middle-aged, and 4% in children were noticed. 50% of cases were observed in AML-M₃ with t(15;17) translocation having male predominance, also good response to therapy and survival periods were reported (Mitelman, 1991). 5% of these cases observed with complete remission was in accordance with the report of Larson et al., 1983, who reported complete remission in disease-free survival cases with t(15;17) abnormality. RT-PCR study reported rearrangement of PML/RAR α chimeric gene rearrangement in younger adults implied high response rates and good survivals period (Vinioú et al., 1995).
32 cases (42%) of AML-M₂ type revealed t(8;21)(q22;q22) with 22 cases (29%) of male predominance over 10 cases (13%) of females. Higher incidence was observed in 18% of adults and 13% middle aged than 10% of females. In Fourth International Workshop, 1984, t(8;21) translocation was reported to be specific for AML, observed in majority of adults and children. Present data from AML-M₂ cases revealed hyperdiploid and normal diploid clones with trisomies, and loss of Y chromosome in one male case. Kita et al., 1994 reported presence of t(8;21) abnormality with hyperdiploid clones having trisomy 5 which revealed favourable prognosis and higher survival rates.

8 cases (10%) of AML-M₅ revealed del(11)(q23) abnormality with 8% of male predominance. Higher incidence was observed in adults (9%). Present study revealed absence of ALL cases in children (<10 yrs). But 11q23 abnormality associated with M₅ type reported by Bernard and Berger, 1995, with intermediate prognosis.

Secondary and additional chromosomal abnormalities observed in therapy related ANLL cases (t-ANLL). Monosomies, deletions, dicentrics, double minutes were frequently observed in AML cases. 3 cases (4%) of AML-M₄ revealed t(6;9). Chromosomal abnormality, observed to have unfavourable prognosis and poor response to therapy. An increased number of marrow basophils associated with t(6;9) in ANLL cases, and an aberrant transcription of a target gene located at 9p34 breakpoint was reported (Von Lindern, 1990).

Higher incidence of +8(20%), +5(17%), -7/7q⁻ (17%) than +4(12%), and +21(9%) were observed in this present study. Reports on +4 with double minutes and del(11)(q23) with t(8;21) after chemotherapy in acute leukemia
cases were highly documented (Roulston et al., 1995), and also suggested a correlation between chromosome pattern and occupation of the patient associated with ANLL. In accordance with these findings, present data also revealed 28% of dicentrics, 8% of rings, and 2% of double minutes in hyperdiploid clones having +4, +5.

3% of AML cases revealed both specific chromosomal abnormality of Ph chromosome, and +5, -7 with poor prognosis. Allen et al., 1992 reported poor prognosis an short survival periods in cases having Ph chromosome and secondary abnormalities. 15% of AML-M3 with t(15;17), +4 revealed good prognosis, and 5% of AML-M2 with t(8;21), +5, -7, dicentrics revealed favourable prognosis.

Present study revealed 7 cases (8%) of death in AML with 6 cases (7%) of death in males. Death was not reported in children. Overall survival period was very short (1-11 months). 4 cases (4%) died within 1 month, 2 cases (2%) within 7 months, and 1 case (1%) in 11 months. Cytogenetically 90% of aneuploid clones were observed, and 10% with insufficient mitosis. Normal diploid clones were completely absent in all dead cases. 50% of cases showed Ph chromosome, -7, dicentris and double minutes.

Good response to therapy and higher rate of survivals were observed in 75 cases (91%) of AML cases, with 4-5 yrs of survival. More than half of the males observed to have good response to treatment modalities. 15 cases (18%) revealed >6 yrs survival and treatment is still continuing. Only numerical chromosomal abnormalities with normal diploid clones and very few trisomies were observed in 4% of AML cases.
5% of cases revealed t(15;17), +5, and +4 chromosomal pattern. Machnicki and Bloomfield, 1990 reported t(15;17) and trisomies greatly increased the survival period of the patient.

**CHRONIC LYMPHOBLASTIC LEUKEMIA (CLL)**

51 CLL cases were reported in this present study. 33 cases (65%) observed in males, and 18 cases (35%) in females. High incidence of occurrence was observed in >35-80 yrs. Occurrence of CLL disease under the age group 1-30 yrs was completely absent in both sexes. Etiology of the disease and incidence of CLL in elders were reported by Conley et al., 1980.

40 cases (78%) of CLL revealed karyotypic abnormalities. Higher percentage of non-random chromosomal abnormalities obtained using polyclonal B-cell mitogens was reported by Gahrton et al., 1980. Present study revealed 21 cases (53%) with numerical chromosomal abnormalities, and 19 cases (48%) with both structural and numerical chromosomal aberrations.

+12 was the only specific chromosomal abnormality detected in 32.5% of 13 CLL cases with a male predominance of 9 cases (22.5%) and 4 cases (10%) of females. An higher incidence of +12 (25%) was observed in elders (>60 yrs) and 7.5% in 30-50 yrs of age group. +12 revealed poor prognosis in majority of CLL cases. 544 cases analysed cytogenetically and by FISH technique revealed +12 defined poor response to treatment and short survival periods (Matutes et al., 1996).
6 cases (15%) of dup(14)(q32) 3 cases (7%) of del(13)(q14), and 4 cases (10%) of monosomy 18 were detected as secondary chromosomal abnormalities found along with hypodiploids, and pseudodiploid clones. Moulds et al., 1996 reported 16% of del(13)(q14) observed by FISH technique in interphase cells out of 400 CLL case study. Retinoblastoma gene (RB) located to 13q14 chromosomal breaks point was suggested as secondary event in the pathogenesis of CLL disease. High incidence of monosomy 18 was also reported by Younes et al., 1994 in CLL cases with intermediate to poor prognosis.

Present data revealed a good survival and response to treatment with normal diploid clones. 10% of CLL cases revealed only normal diploid clones with >5 yrs survival period.

Death in 8% of 4 CLL cases occurred within 6 months to 2 yrs. 6% of cases showed +12, 14q' and dicentrics, died with in 6 to 9 months (shortest survivals). 2% of cases showed pseudodiploids and hyperdiploids with +12, and -18, died within 1-2 yrs. Juliusson et al., 1993 reported +12 with other secondary chromosomal abnormalities had adverse prognosis associated with advanced disease and aggressive clinical course.

47 cases (92%) of CLL revealed intermediate prognosis, under clinical course of treatment. 63% were elders and 29% were adults. 10% of 8 cases showed only normal diploid clones with a good survival period of 4-5 yrs and good response to treatment modalities. Juliusson et al., 1993 reported good prognostic indicator of patients with only normal diploid clones.
The multistage process of carcinogenesis and mutagenesis increased the incidence of cancer, and exhibited genetic alterations which were always associated with chromosomal translocations and rearranged oncogene(s). This was highly exploited in all the four types of leukemias (CML, ALL, AML, CLL) with specific chromosomal translocations associated with rearranged oncogenes and extensively accepted for the diagnosis of cancer.

The progression of leukemic disease was expressed by the presence of secondary and additional chromosomal abnormalities, which eventually noticed with poor prognosis and response to treatment; reduced the period of survival and even fatal to some extent.

Aneuploidy nature of cancer cells were focussed by valuable flow-cytometric analysis, and the association of chromosomal translocations and the specific oncogene(s) mapped to its break points were highlighted with molecular genetic approaches by using highly sensitive PCR and RT-PCR methods.

Present observations from the correlative study of cytogenetics, flow-cytometry and molecular genetics not only useful in diagnosis of the disease but also highly valuable for evaluating the prognosis of the disease and also to monitor patient's response to therapy.