Introduction
The term 'Preleukemia' was introduced by Block et al. (1953) to include various clinical entities that harbour a high risk to develop leukemia. Preleukemia (PL) refers to diseases of haematopoietic dysfunction that precede acute myeloblastic leukemia (AML) by durations extending over months or years. According to Linman and Bagby (1978), PL cannot be attributed to specific haematological disorder of known etiology and pathogenesis.

Preleukemia is a marrow stem-cell disorder associated with clinically observable haematological abnormalities which may later develop into acute myeloid leukemia (AML) over a period of time (Linman and Bagby, 1976, 1978). It is a clonal stem cell neoplasm marked by hypercellularity of bone marrow with peripheral blood cytopenia and ineffective haematopoiesis. A large number of disorders are included under PL and may consist of genetically determined syndromes like Fanconi anemia, Down syndrome, Bloom syndrome, and conditions such as myeloproliferative disorders, aplastic anemia and myelodysplastic syndromes (Kleihauer, 1980).

The individuals with Down syndrome (DS) are cytogenetically characterised by trisomy 21 in most of the cases and rarely by a translocation involving chromosome 21 or by mosaicism. Patients with Fanconi anemia and Bloom syndrome are characterised by chromosomal fragility and possess structural aberrations of chromosomes. In patients with Fanconi anemia (FA), the aberrations involve nonhomologous chromosomes and in Bloom syndrome,
chromosomal interchanges are between homologous chromosomes. Chromosomal aberrations may also be present in myeloproliferative disorders, aplastic anemia and myelodysplastic syndromes (MDS) or these may be due to genetic defects at molecular level. It is thus evident that the cells of preleukemic patients suffer from an insult to the genetic material either at the level of chromosomes or at the molecular level.

This thesis is a presentation of the details of a study carried out to examine cytogenetic aspects of Down syndrome, Fanconi anemia and myelodysplastic syndromes employing cultures of lymphocytes from these patients, and to examine molecular defects in a few cases of myelodysplastic syndrome.

DS, previously referred to as mongolism, is a complex pathological condition with a wide spectrum of phenotypic features. It is the most common cytogenetic cause of mental retardation. The reported incidence of DS in general population is more than one per thousand newborns. The frequency of DS is relatively more in children born to older women, that is, those over the age of 35 years. DS individuals are known to be at an increased risk of developing leukemia compared with those of healthy children. The incidence is 15 to 20 times higher than in normal children (Smith and Berg, 1976).

The cause for an increased incidence of leukemia in DS remains to be elucidated. Transient leukemia and leukemoid response occur frequently in newborn DS cases. It is known that the unusual haematologic response in DS infants may be due to altered function of gene or genes located on chromosome
21, leading to altered hemopoiesis and thus may predispose the trisomic children to leukemia (Smith and Warren, 1985). Non random chromosomal changes are considered to be important events in cancer and there are reports on the involvement of chromosome 21 in these changes. Trisomy 21 is a common feature not only in acute lymphoblastic leukemia, but also in acute myeloid leukemia (Heim and Mitelman, 1995).

Lymphocytes of DS patients are highly sensitive to ionizing radiation as indicated by the frequency of induced chromosomal aberrations (Shafik et al., 1988). According to Tadeshi et al. (1990), chromosomal breakages seen in DS lymphocytes on irradiation and without selection, are confined preferentially to fragile sites. From this observation they inferred that the action of radiation on DS lymphocytes was site-specific and that this was probably determined by heredity. A study carried out in this direction (Appendix I) suggested that the breakpoints in DS lymphocytes exposed to radiation were non-random. This inference needs to be established employing a large number of samples and testing with different doses of radiation.

Two important cytogenetic parameters employed to assess mutagenicity of a particular agent are chromosomal aberrations (CA) and sister chromatid exchanges (SCE). Though both of them are indicators of insult to the chromosomes, different mechanisms are known to operate in their induction. Contrary to the fact that most chemical mutagens elicit a dose dependent increase in SCE, radiation does not induce such a response. The irradiated fibroblasts and lymphocytes of DS individuals are reported to exhibit
increased incidence of chromosomal aberrations (Higurashi and Cohen, 1972). Two fold increase in the frequency of SCE in trisomic cells was reported by Heidemann et al. (1983) compared with that in normal diploid cells. Krishnan (1986) also observed a similar increase in SCE. It would be of interest to investigate the incidence of SCE in lymphocytes of DS patients and to compare that with the recorded frequency in diploid cells.

There have been attempts by several investigators to study the modulatory action of a variety of chemicals on radiation-induced cytogenetic damage. 2-deoxy-D-glucose (2-DG), a glucose antimetabolite, was tested by Jain and coworkers in yeast cells (Jain et al., 1977b) and later in mammalian cells (Jain et al., 1979) to examine its modifying action on radiation-induced genetic damage. They demonstrated the possibility of employing 2-DG as an adjuvant in the radiotherapy of cancer. Girijamani and Gopinath (1997) have suggested the possibility of employing 2-DG as an antimutagen. In this context, it was thought desirable to examine the action of 2-DG on chromosomal aberrations in trisomic cells, namely DS lymphocytes, and thus to supplement the data generated by Girijamani (1988).

Fanconi anemia, an autosomal recessive disorder, is characterized by fragility of chromosomes. The patients suffering from FA are highly predisposed to malignancy. The main clinical features observed include hyperpigmentation, skeletal and urogenital malformations and small stature. Haematological disturbances encountered are pronounced reduction in the number of erythrocytes, lymphocytes and thrombocytes.
Endogenous chromosomal breakage found associated with FA (Schroeder et al., 1964; Swift and Hirschorn, 1966) is considered responsible for increased incidence of spontaneous chromosomal aberrations. While, there have been extensive investigations to record the incidence of structural abnormalities of chromosomes, there have been only a few attempts to assess SCE in FA lymphocytes. Nazarenko and Burmakina (1984) reported increased incidence of SCE in FA cells, compared with that seen in the lymphocytes of healthy individuals. There is need to examine the frequency of SCE in FA lymphocytes and also to record the incidence of structural aberrations of chromosomes and to identify different types of aberrations.

Myelodysplastic syndromes comprise of heterogeneous haematologic diseases in which an initial mutation of a pluripotent haematopoietic stem cell gives rise to abnormal control of cell proliferation and differentiation (Gadner and Hass, 1992). Disease progression being a multi-step process, involves clonal expansion leading to establishment of abnormal clones and suppression of normal haematopoiesis. Peripheral blood cytopenia and normo-to hypercellular bone marrow constitute the haematological picture in MDS patients. The disorders that make up MDS range from the relatively indolent refractory anemia without excess of blasts (RA) and refractory anemia with ringed sideroblasts (RARS), to those that are more aggressive, like refractory anemia with excess of blasts (RAEB) and refractory anemia with excess of blasts in transformation (RAEB-t) (Cheson, 1992). Chromosomal analysis in them was considered by Yunis et al. (1986) to be an independent prognostic indicator.
According to Mori et al. (1992), though the mutational inactivation of p53 gene is infrequent, it plays an important role in bringing about tumorigenesis in several types of neoplasms. Employing techniques such as PCR-SSCP and sequencing of nucleotides, Adamson et al. (1995) studied point mutations in exons 5-8 of p53 gene of MDS patients. From the observations, p53 mutation was inferred to be a terminal genetic event in the evolution of MDS. It is of advantage to subject bone marrow aspirates of MDS patients in our population to chromosomal analysis with a view to find whether there exist cytogenetic anomalies. Further, cells of MDS patients in our population also need to be examined for the presence of genetic defects employing molecular techniques.

In the light of the above, it was thought desirable to carry out a study on PL, with special reference to the cytogenetic evaluation of DS, FA and MDS, and also to examine MDS cases employing molecular techniques. Following were the objectives of the study thus executed.

1. To determine the frequency of chromosomal abnormalities in DS lymphocytes on exposure to different doses of gamma rays (1 Gy and 3 Gy) and to record different types of structural changes.

2. To examine the action of 2-DG on radiation-induced chromosomal aberrations in the lymphocytes of DS individuals.
3. To determine the level of SCE in DS lymphocytes, with a view to examine whether trisomic cells differ from normal diploid cells in the expression of SCE.

4. To record various breakpoints encountered in DS lymphocytes on exposure to radiation, non-random breakpoints in particular, with a view to find out whether there exists correlation between them and cancer-specific breakpoints, rare fragile sites and oncogenes.

5. To assess the frequency and types of spontaneous chromosomal aberrations in FA lymphocytes.

6. To compare the frequency of SCE in DS and FA lymphocytes.

7. To analyse chromosomal aberrations in MDS patients, and

8. To employ PCR-SSCP analysis to determine point mutations in exons 5-8 of the p53 gene in MDS patients.