Summary and Conclusion
6. SUMMARY AND CONCLUSIONS

The present study comprised of patients suffering from 'preleukemic conditions' namely, Down syndrome (DS), Fanconi anemia (FA) and myelodysplastic syndrome (MDS). DS patients were referred to the Department of Genetics, Institute of obstetrics and Gynaecology, Egmore; to Pathway, Thiruvanmiyur and to the Department of Genetics, Dr. ALMPGIBMS. On the other hand, patients diagnosed to have FA and MDS were referred to the Departments of Haematology, Institute of Child Health, Egmore and Government General Hospital & Chennai Medical College, Chennai, respectively. All these institutions are located in the city of Chennai.

Cytogenetic investigations were carried out using PHA-stimulated leucocyte culture (Hungerford, 1965) technique in case of DS and FA patients. Direct chromosome preparation technique from bone marrow aspirates was employed to determine clonal abnormalities in the study of MDS cases. Chromosome aberrations were detected using trypsin-Giemsa (GTG) banding technique (Seabright, 1971) and the breakpoints were designated according to the standard nomenclature (ISCN, 1995). The staining technique for sister chromatid differentiation was modified from that of Goto et al. (1975). Polymerase chain reaction (PCR) - Single strand conformation polymorphism (SSCP) analysis technique was employed to detect mutations in exons 5-8 of p53 gene in a few cases of MDS.
Radiosensitivity of DS lymphocytes (using two doses of gamma-radiation), the modulatory effect of 2-deoxy-D-glucose (2-DG) on radiation-induced damage, and the frequency of sister chromatid exchanges (SCEs) were assessed in DS patients. A correlation of the non-random break points involved in chromosomal rearrangements seen in irradiated lymphocytes with the sites of location of cancer-specific breakpoints, oncogenes and rare fragile sites was also carried out. The levels of spontaneous chromosome aberrations and SCEs were evaluated in FA patients.

Irradiation of DS lymphocytes with gamma rays at G₀ stage revealed an increased number of aberrant cells. A two-fold increase in the percentage of metaphases with aberrations was observed at the higher dose (3 Gy). The different types of aberrations seen were gaps, breaks, fragments, dicentrics, rings, exchanges and double minutes.

A reduction in the frequency of chromosome aberrations was observed when irradiated lymphocytes of DS patients were subsequently treated with 2-DG which is an antimetabolite of glucose.

The frequency of spontaneous sister chromatid exchanges (SCEs) in DS patients was noted to be lower than the laboratory control data. However, this observation needs verification on a larger sample size.

Twenty-nine breakpoints involved in chromosome aberrations recorded in irradiated (3 Gy) DS lymphocytes were found to be non-random, that is, they were detected in at least two patients. However, at the lower dose
of 1 Gy, only four breakpoints were seen to be non-random. With the exception of three breakpoints, the remaining were found to correlate with location of cancer-specific breakpoints. While eight of these bands coincided with those in which oncogenes have been localized, five were noted to be sites of rare fragile sites. The increased radiosensitivity as evidenced by metaphases with chromosome aberrations may result in stable chromosome rearrangements which in turn, may lead to an increased incidence of leukemogenesis.

An increased incidence of spontaneous chromosome aberrations seen in cultured lymphocytes from FA patients was consistent with the diagnostic criteria for this chromosome fragility disorder. However, an inter-individual variation in the percentage of aberrant metaphases was noted. Gaps, breaks, fragments, dicentrics, rings and exchanges constituted the different types of aberrations recorded.

Direct chromosome preparation technique from bone marrow aspirates, attempted in a limited number of MDS patients, revealed a normal karyotype.

Single strand conformation polymorphism (SSCP) analysis of amplified DNA products from nine MDS patients to detect mutations in exons 5-8 of p53 gene was carried out. In one patient a mobility shift of exon 8 amplified DNA was observed indicating a point mutation. This patient was an elderly woman who was diagnosed to have refractory anemia without excess blasts (RAWEB). This is an early MDS type when compared to advanced subtypes such as RAEB, RAEB-t and AML from MDS. This observation
suggests an extension of such studies to a large number of patients (of all subtypes) to determine the role of p53 in the causation of MDS, in particular, disease progression and evolution to AML.

CONCLUSIONS

1. Lymphocytes of patients with Down syndrome exhibited increased levels of aberrant metaphases upon exposure to gamma rays. Increased radiosensitivity was observed at the higher dose employed.

2. 2-deoxy-D-glucose, an antimetabolite of glucose, caused a reduction in the frequency of radiation-induced damage in irradiated DS lymphocytes.

3. Due to paucity of samples the observation of a lower frequency of SCE in DS lymphocytes could not be confirmed. An extensive study needs to be carried out.

4. The study of correlation of non-random break points detected in irradiated DS lymphocytes with cancer-specific breakpoints, oncogenes and rare fragile sites may throw light on the mechanism underlying predisposition of DS patients to leukemia.

5. Fanconi anemia patients exhibit an increased level of spontaneous chromosome aberrations although inter-individual variations may be encountered.
6. A slightly increased frequency of SCE was seen in FA patients as compared to that observed in DS patients. This study requires to be extended to include more number of FA patients and to those diagnosed to have other chromosome fragility disorders for a comparison.

7. Direct chromosome preparation method revealed a normal chromosomal karyotype in all the patients with myelodysplastic syndrome subjected to cytogenetic investigation.

8. PCR-SSCP analysis revealed a point mutation in exon 8 of p53 gene in one of nine patients with MDS. This patient was in an early stage of the disease (RAWEB) indicating the importance of extending such studies to all subtypes of MDS.