CHAPTER-VII : SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The rapid development of human genetics during the past couple of decades and the discovery of numerous cytogenetic abnormalities have opened up new aspects of human genetics. It has made remarkable progress and momentousness show no sign of abatement. In recent years, study of chromosome aberrations and molecular aspects of genetics have steadily gained importance not only for the genetics but also for the entire field of Medical Genetics, this being the result of the discovery of culture methods by which human chromosome could be made visible by differential staining techniques.

Chromosome abnormalities can be divided into those which involved the sex chromosomes and those which involve the autosomes. In the present study these can be further subdivided into numerical and structural abnormalities.

(a) Numerical abnormalities

It is the aberration of chromosome number (aneuploidy) such as XX/XY +21; XX/XY +15; XX/XY +18; XX/XY -20 and mosaicism with normal cell line 46,XX/XY; and 47,XX/XY + (21 or 15 or 18) chromosomes in case of autosomes. In the sex chromosome numerical anomalies were found in the form of XO, XXY, XXX etc. mosaicism X/XX, XX/XXX, XX/XY, XX/XXY etc.

(b) Structural aberrations

Structural aberrations in form of translocation deletion, inversion, ring formation isochromosome etc. in individual chromosome. In the present

study, D/G translocation, balanced Robertsonian translocation, iso(Xq) were found. About 75% of the trisomy cases were caused by primary non-disjunction and there seems to be no sex predilection.

The thesis consists of Volume-I, Chapter-I which includes Introduction, Objectives, Review of Literature and Material and Methods. In the beginning introduction to cytogenetics has been given. Main objectives of the present study have been elaborated. This chapter includes review of different studies conducted by various investigators at different parts of the world. By going through the review one would get the idea regarding the significance of chromosomal complement in the phenotypic and genotypic development of individuals, the defect in either physical, mental or sex organ development, caused by the chromosomal aberrations and its incidence in general populations. Many investigators have elaborated the consequences of the abnormalities faced by the individual. Attempts have been made to include ancient thinking to the recent modern era of molecular genetics to understand the mechanism and cause of chromosomal aberrations under this chapter.

Different views of number of investigators regarding the functional significance of autosomes, X and Y sex chromosomes have been explained. Ideas about gradual development of various staining techniques including fluorescent staining, G T G banding, C-banding are mentioned. The role of sex chromosomes that is X and Y chromosomes in development of the gonad, particularly the role of Y chromosome in the differentiation of the gonad into the testis and the region involved in this process is discussed. Different genes, located on X chromosome, controlling the biological functions are described. Mapping of gene(s) on X and Y chromosomes are also illustrated. The hypothesis on H-Y antigen as well as testicular differentiating factors (TDF) region of Y-chromosome which is supposed to trigger the differentiation of gonads into the testis is described. The normal and abnormal role of X and Y chromosomes in the phenotypic and genotypic development of individual is explained. The work done by number of investigators on various manifestations of chromosomal abnormalities in the clinically suspected individuals in

Down's syndrome, Edward's syndrome and autosomes mosaicism, primary amenorrhoea, Turner's syndrome, Klinefelter's syndrome, sex chromosome mosaicism, hermaphroditism, ambiguous genitalia, testicular feminization syndrome, abnormal sexual development with normal chromosome, agonadism, cytogenetic study in haematological malignancies are summarised with clinical features and cytogenetic findings.

Chapter-2 : includes the Materials and Methods used during this study. Improved research techniques have enabled us to understand the manifold process involved in somatic and sexual development. The techniques involved in vitro culture of peripheral blood and bone marrow cells in different media in the presence of phytohaemagglutinin (PHA) followed by treating dividing cells with a very weak salt solution (hypotonic), it is possible to spread out the metaphase chromosome to their genetic make up. The karyotype of a female individual usually consists of paired sex chromosomes while that of a male individual of unpaired sex chromosomes, that is 46,XX and 46,XY respectively. Once the sex chromosomes have been identified, true genetic sex of an individual could be determined. An individual with additional or deleted autosomes or sex chromosomes could be identified to know the genetic cause for abnormal phenotype or genotype features. Different staining, viz. X and Y chromatin, Giemsa staining , GTG, C, Q, NOR banding techniques are described. In addition to the peripheral blood culture procedure of bone marrow culture, with some modifications, for haematological malignancies is described.

Chapter-3 of Volume-II, Part-I to Part-III includes results/observations obtained during the study. Cytogenetic study is undertaken on 629 patients referred for autosomal and sex chromosomal abnormalities. Out of 629 cases, 200 cases (Part-I) are referred for Down's syndrome, 11 cases of other suspected chromosomal abnormalities. 219 cases are comprising of 88 females and 131 males. Out of 200 cases, 62 are 47,XX/XY +21, Down's syndrome; 16 mosaic Down's; 4 trisomy-18; 2 trisomy-15 and one monosomy F group. Other 418 cases (Part-II) are of clinically suspected sex chromosome abnormalities, which include primary amenorrhoea, Turner's stigmata, Klinefelter's syndrome, repeated abortions, Mullerian agenesis,

ambiguous genitalia, hypospadias and hypogonadism, delayed puberty, Robertsonian translocation, testicular feminizing syndrome. Out of 418 cases, 228 are females and 183 males. Out of 418 cases studied, 12 patients were found to have Turner's syndrome. Out of these 3 had 45,X karyotype, 6 had X/XX mosaicism, one had 45,X; 46,X + marker chromosome; two showed 46,X iso(Xq) chromosomal complements, 4 with triple X syndrome.

Thirteen male patients were referred for Klinefelter's syndrome and all of them had 47,XXY karyotype. Testicular feminization was found in 3 cases. In one case balanced Robertsonian translocation was found in a female with spontaneous abortions. In 5 cases, though the chromosomal complements were normal i.e. 46,XY and 46,XX; 3 had absent gonads and 2 cases found to have ovotestis on one side and ovary on other side. Forty cases were referred for evaluation of genetic sex; 46,XY found in 29 cases and 11 had a 46,XX female karyotype.

Hormonal levels were studied in certain cases of different groups such as spontaneous abortions, primary amenorrhoea, Mullerian agenesis, azoospermia, ambiguous genitalia, hypogonadism, Klinefelter's syndrome, sterility, testicular feminization syndrome, primary ovarian failure. Hypogonadotrophic hypogonadism and primary testicular failure were seen in hypogonadism and in delayed puberty cases. Histopathological studies were conducted from gonadal biopsy in selected cases.

Part-III includes 40 cases for haematological malignancies were studied by bone marrow culture. Complete clinical history, histological aberrations, therapy and prognosis were obtained and summarised.

The last **Chapter-4** of Volume-II of Discussion focussed on the results obtained during the study and were discussed and compared with the recent literature. Various groups discussed include Down's syndrome, Trisomy-18, Trisomy-15, primary amenorrhoea, Turner's syndrome, Mullerian agenesis, primary sterility, Klinefelter's syndrome, azoospermia, oligospermia, repeated abortions, ambiguous genitalia, delayed puberty,

hermaphroditism, X/XX and XX/XXX mosaicism, marker mosaic, X isochromosome, testicular feminization syndrome, Robertsonian translocation, XY agonadism. Their clinical features, cytogenetic findings, histological findings hormonal levels and their incidence in population were discussed and compared. In Part-III, cases of haematological malignancies, which includes chronic myeloid leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, acute lymphocytic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, multiple myeloma were discussed and compared with recent literatures, to find out correlation between cytogenetic findings and prognosis of the diseases.

PART-III : HAEMATOLOGICAL MALIGNANCIES

A prospective study of 40 consecutive cases of different haematological malignancies admitted or referred to medical units is presented. All the cases were subjected to cytogenetic analysis after complete clinical, haematological and/or histological staging. The chromosomal changes were compared with the type of haematological malignancy, prognosis and progression of the disease. Cytogenetic data so collected was analysed to derive conclusions after critical evaluation of the observations in light of the review of available literature.

I. Methodology

(i) Bone marrow aspirate culture was found to be very convenient, reliable and quick method for the cytogenetic analysis of the patients with haematological malignancies.

(ii) The collecting medium used in the present study was lactated Ringer's solution instead of costly nutrient media or addition of serum. This switches over to a cheaper and indigenous alternative did not vitiate the cytogenetic findings in any way. Results with this collecting media were found to be comparable in quality, spread and number of metaphases with any other media.

(iii) Peripheral blood culture was tried in 4 patients with high peripheral blood blast cell count (more than 30%) and dry bone

marrow tap. For unexplainable reasons, none of the four cultures was successful. Later on, the bone marrow trephine biopsy piece gave satisfactory result. The obvious preference in the present series was for the bone marrow biopsy piece instead of peripheral blood culture.

(iv) Flame dried technique for the preparation of slides gave results as good as air-dried method and was preferred in the study.

(v) G-banding was tried in all the patients, but the results were not satisfactory.

(vi) The chromosomal preparation obtained with the standardised 90-120 minutes colchicine treatment of fixed concentration was found to be very variable. This inconsistency was seen not only in different patients but also in different fields in the same patient. This suggests different susceptibility of cells in varied phase of cell maturation and division.

II. General

(i) The present study of total 40 cases comprised of 12 cases of CML, 3 cases of CLL, 4 cases of AML, 3 cases of ALL, 10 cases of HL, 6 cases of NHL and 2 cases of MM.

(ii) Cytogenetic study was successfuly carried out in 36 out of 40 cases with failure in only 4 cases (10%) - a comparable rate.

(iii) Of the 4 patients who could not be karyotyped, 3 had Hodgkin's lymphoma and 1 had acute lymphocytic leukemia.

(iv) Out of these 4 patients, the failure in 2 patients, 1 each of ALL and HL was due to no observable metaphases; whereas, the remaining 2 patients had very poor chromosomal spread.

(v) Both the patients with no observer metaphases had markedly advanced disease. One of the supposition is the probable relationship of failure in obtaining metaphases with advancement of the disease.

CONCLUSIONS

This study deals with various types of autosomal aneuploidy - trisomy 21, trisomy 18, trisomy 15, human sex abnormalities and chromosomal disorders; which form a vital part in the area of medical genetics.

1. The results obtained by studying cytogenetic abnormality in different abnormal phenotypic features and in suspected chromosomal aberrations in haematological malignancies, ambiguous genitalia and spontaneous abortions are encouraging and justify the necessity of this study in clinically malformed individuals.

2. The results of this study helped to confirm the chromosomal abnormalities in clinically suspected cases and ultimately further management of patients.

3. The present study had enabled us in understanding the pathogenesis and numerous chromosomal aberrations in human beings.

4. From the results of the present study, genetic counselling has been extended to the members of the family of proband and couples who were at a high recurrence risk, for the future progency.