CHAPTER-VI : DISCUSSION

DISCUSSION

PART-I

With the modifications and advancement of the techniques in human cytogenetics, our knowledge about the chromosomal abnormalities and their subsequent effect on the genotypic and phenotypic development of individual, has increased manifold, to our revealing mind. In the last decade and half has witnessed human progress in finding out genetic cause for number of diseases in the affected members and amongst the other family members.

Because of the tremendous understanding of the chromosomal abnormalities it has been shown that the fraction of individuals, with such abnormalities seen at birth and in early life is comparatively small, as compared to the substantial fraction of fetuses which are eliminated during early phase of intrauterine life as spontaneous abortions.

Number of studies have been conducted on various populations in different parts of the world to understand the chromosomal abnormalities and their subsequent effect on the affected individual.

According to the data, 0.63% of the new borns have chromosomal abnormalities. In that, frequency of Down's syndrome is 1:800; of these in 95% cases, there is trisomy 21 and about 4% of the individuals show translocation, while 1% cases are mosaic (Borgaonker, 1989). Out of these 0.63%, 33.6% have sex chromosomal abnormalities. The incidence of chromosome aberrations in spontaneous abortions is as high as 48%. Of these abnormalities, 20.5% were numerical aberrations of the sex

chromosomes. In primary amenorrhoea, chromosome aberrations are present in 23.5% of cases (Borgaonker, 1989).

The incidence of Down's syndrome at conception is much greater, but more than 60% are spontaneously aborted, and at least 20% are still-born. The incidence increases with increasing maternal age. Thus, the incidence at the 16th week of pregnancy is 1:200 for a 36 year old mother, rising to 1 in 100 at 39 years and 1 in 50 at 42 years. The age-related birth incidence is approximately 30% lower, due to spontaneous miscarriage and appears to be approximately three-fold higher during first trimester, at the time of chorionic villus sampling, than at birth (Emery, 1988).

In the present study, out of 219 patients with clinical symptoms of mental retardation, delayed mile stones and Down's syndrome, 62 patients of both sexes showed 47,XX/XY +21 (28.30%), 16 patients with 46,XX/XY | 47,XX/XY +21 (7.30%) mosaic. The frequency of trisomy 21 and mosaicism was comparatively high in the present series, which could be attributed due to most selective group of patients for cytogenetic studies. However, Verma et al (1977) reported that frequence of severe mental retardation as 0.67% per 1000 population. The frequency of Down's syndrome is reported 1.5 per 1000 births and 0.8 for all India series.

The fact that the majority of the mongoloid patients are born to older mothers led to assumption that the majority of the cases have a relation to maternal aging. As more cytogenetic data accumulated, including the present study, however, it became apparent that G-21 trisomy is not confined to children born to older mothers, but occurs among children born to younger mothers as well. While the presence of a supernumerary G-21 chromosome is not the only type of chromosomal findings in mongolism, it seems to be the most frequently observed one amongst mongols born to mothers of all ages. In the present study, the maternal age of these patients found below 24 years showing the involvement of a children of young mothers (57.50%).

Other people like Polani et al (1960) - 66%; Makino et al (1960) in Japan and Hamerton et al (1962) in England, reported 80% and 85% mongols respectively, who were born to young mothers. Lehman et al (1962) in Sweden considered 24 years as the upper age limit for young mothers to have mongols. In their study, they found 97% mongols borned to young mothers which are similar to the findings in the present study (57.50%).

The observation of a fairly large number of standard G-21 trisomies among children born to young mothers indicates that either the mechanisms thought to explain meiotic non-disjunction in older mothers are not so much age-specific or that other mechanisms can produce G-21 trisomy as well. Referring data of past studies tend to support the latter possibility as the major factor.

The following mechanisms or factors may be considered to be causative in this respect.

(1) One of the parents may be a mongol having the G-21 trisomy and as the result of secondary non-disjunction half of her/his gametes will carry a supernumerary chromosome which, if fertilized by a normal gamete, produces a trisomic mongoloid infant. In the present study, a few couples who were suspected as carriers were found to have normal male and female karyotype. Most cases of G-21 trisomy arises from non-disjunction usually at first but sometimes second meiotic division. Overall, the mother contributes the extra chromosome in 80% of cases and the father in 20%. One reason for this may be reduced fertility of mongoloid males (Rehn et al, 1957; Levan et al, 1960; Forssman et al, 1961; Hanhart, 1960b; 1961; Thuline et al, 1961; Goodman, 1961; Johnston et al, 1963; Gustavson, 1964).

Family pedigree studies by M.Rangoonwala (1990) clearly showed that the evidence from the data presented that incidence of maternal meiotic-I, non disjunction were in more than 50% cases. Maternal meiotic-II was in less than 20% cases. On the other hand, paternal meiotic-I was found in less than 15% of cases and paternal meiotic-II in 10% cases.

Parental origin of non-disjunction was more in maternal meiotic-I than in meiotic-II. In many cases, in our studies, showed that some of the mothers had undergone radiation and some were using oral contraceptive pills regularly, which may possibly have aggravated in to non-disjunction in normal mothers.

(2) Second possibility to have a Down's syndrome child in the parents with normal karyotype may be a mosaic for G-21 trisomy with trisomic cells in the gonads. This results in the secondary non-disjunction. Polani (1963) reported all the tissues, except the gonads, composed to normal cell lines, while trisomic cells were present only in the gonads. Phenotypically such persons may be free from the main stigmata of mongolism and therefore, may have excaped detection, in the present study but they can produce gametes with supernumerary chromosomes. Secondary non-disjunction in mosaic was reported by Blank et al (1962); Smith et al (1962); Edwards et al (1963) and Weinstein et al (1963).

(3) Third factor would be the environmentally produced non-disjunction in parents with normal chromosome complements lead to the production of gametes with supernumerary chromosomes. In our studies, it was recorded that some of the mother had undergone radiation exposure and some were using oral contraceptives. It has generally been assumed that radiation was one of the causes of Down's syndrome, which was independent of maternal age (Penrose et al, 1966).

(4) Familial concentration of mongolism; could also be considered, was studied by Hamerton et al (1961) in England, Forssman et al (1962) in Sweden, Warkany et al (1964) in U.S.A. and Soltan et al (1964) in Canada. In light of the above findings and in view of the fact that many different chromosomal observations are associated with one another in the same families (Hecht et al, 1964); the existence of a genetic predisposition to chromosomal errors has been postulated. The predisposition could be one of the factors in normal parents with Down's children in the present study.

(5) As mentioned earlier, the occurrence of non-disjunction during the first meiotic division (first cleavage division) of the zygote can result in the production of two cells, one trisomic for G-21 and the other monosomic for the same. Since autosomal monosomy has not been observed in man, it is supposed that cells monosomic for G-21 die and the individual developing from the trisomic product of a meiotic non-disjunction survives.

Since fertilization occurs after moisics-I in the ovarian and since most maternal errors appear to have occurred in moisosis-I, those hypothesis involving delayed fertilization and fallopian tube malfunction can be eliminated as major factors.

Many studies have reported high frequency of maternal meiosis-I errors (Bricelli et al, 1989; Hassold et al, 1984; Juberg et al, 1983) and it is virtually certain that non-disjunction at this stage is the most common source of trisomy 21 in the Down's patients of the present series. However, parental origin of trisomic-21 chromosome, can be attributed only in about 50% to 80% of fetuses or children with Down's syndrome using cytogenetic markers.

In the present study, in the male, 50% patients with trisomy-21, flat bridge of nose, narrow high arch palate and mongoloid slant were the most common phenotype abnormal features found. Similar findings were reported by Smith (1964). Other features like simian crease, furrowed and protruding tongue, ears deformity/abnormality and mental retardation found in between 30% and 40% patients, while Smith (1964) observed these features in more than 50% of patients.

In the present study, most specific other feature in male, mongol was the undescended testes and small size penis. Same was reported by Smith (1964).

Epicanthus, flat occiput, short crooked 5th finger and increase distance between big toe and second toe were the next features found common in

the patients. Smith (1964) observed these anomalies in more than 50% of Down's patients, while less than 30% of the patients were found to have above abnormalities in the present study.

In the present study, mongols found to have a lower birth weight and a lower weight during they grow. They are shorter than normal. Same findings were observed by Parker (1950), Smith et al (1955) and Oster (1953).

In the present series, female mongols were found to have high percentage of flat bridge of nose (54.50%), mental retardation (59.0%) and delayed mile stones (50%) in comparison to male mongols with 50%, 32.50% and 27.50% respectively. However, higher involvement of specific sex in Down's syndrome is not found in the literature.

Dermatoglyphic pattern in Down's syndrome in the present study found to have ulnar loop in 65% and whorls in 28.25% of the fingers. Simian crease were observed in 65.40% of cases; 'atd' angle found to be wider. Similar findings were cited by Niyogi and Srivastava (1986) - 60% ulnar loops, high percentage of whorls and more than 50% cases with simian crease.

Turner's Syndrome : PART-II

In the present study, among the 12 cases with the clinical diagnosis of Turner's syndrome and Mullerian agenesis, 3 cases showed 45,X. Their main phenotypic features were short stature (125 cms to 138 cms; age 13 to 18 years), absence of secondary sexual characters and primary amenorrhoea. Other complaints like mental deficiency, coarctation of the aorta and colour blindness were not observed in these patients. The average height of 3 patients was 130 cms, while Rodens et al (1984) recorded height range from 133 to 153 cms. The standard for height may be varies from race to race and country to country. In early childhood growth of body is normal but by late childhood the ratio of setting to standing height is frequently increased suggestive of retardation in growth of the legs (Lippe, 1982). The span is found to be greater than height in the present study, as also by Albright et al (1942). While Rodens et al

(1984) suggested final height and average parental height relations. The etiology of the short stature is still not clear but it is definitely believed that it is not because of the deficiency of growth hormone (Kaplan et al, 1968) somatomedin (Saenger et al, 1976) or adrenal or gonadal steroids (Sklar et al, 1981).

During prebanding era, Gordan et al (1955) reported retardation of growth from birth and the patient may go on growing until 21 years of age. Some experienced a sudden cessation of growth at the time of appearance of scanty pubic and axillary hair (Albeuse-Fernet et al, 1955). Some investigators believe that short stature is genetically determined (Graber, 1937; Varney et al, 1942). Other consider it to be a developmental defect like dysgenetic gonads (Willians et al, 1944), while a few maintain that it is due to estrogen deficiency as estrogen treatment may promote further growth in these patients (Graber, 1937; Albright et al, 1942; Grumbach et al, 1955; Hauser et al, 1956). The growth abnormality may reside in response to the chondrocyte to somatodins.

Two patients of the present study did not menstruate. They have gonadal dysgenesis and infantile internal genitalia. Ferguson-Smith et al (1964) reported spontaneous menstruation in 7 of 83 cases of 45,X studied. However, it was not known whether the menstrual cycles were ovulatory or anovulatory. Out of 83 gonads of 24 patients were examined, 2 were showing ova. It is possible that some 45,X patients enter puberty with a few primordial follicles, but more likely is the possibility that patients with spontaneous menstruation are mosaic individuals and have an undetected normal cell line.

It is now well known that two active X-chromosomes are required in human for the normal development of oogonia and oocytes. Until the 3rd month of gestation, no appreciable differences were noted between gonads of 45,X and XX fetuses; after the 3rd month an increase in connective tissue stroma and impaired formation of follicles were found. Thus, primordial germ cells seed the primitive gonad in 45,X individuals, but may degenerate during oocyte formation and folliculogenesis, and the

surviving oocytes undergo accelerated atresia (Weiss, 1971). The oocytes degenerate shortly after the formation of the primary follicles (Jirasek, 1976). The presence of few follicles in the gonadal streaks in 45,X infants is probably common at birth but rare by late childhood and adolescence.

Hormonal essay in the three patients with 45,X in the present study is suggestive of chromosomally incompetent-primary ovarian failure. Cytogenetic, clinical and gynaecological analysis in a series of gonadal dysgenetic patients and their families were studied by Mattern et al (1971). The study was conducted on 46 individuals; 16 had a karyotype of 45,X, while 15 found to be mosaics. A study of 78 cases of clinically and cytogenetically proved Turner's syndrome by Phadke et al (1975) in Poona, over a period of 3 years, 16 (20.5%) were found to be Turner's mosaic.

Cytogenetic findings in 89 cases of Turner's syndrome were reported by Schmid et al (1974) which include 61,XO, 8,X/XX, 1,X/XY and 19 cases of structural aberrations.

The clinical, cytogenetic and dermatoglyphic studies in 100 families with Turner's syndrome carried out by Palmer et al (1977). They found 60% had 45,X, 10% X,XX or multiple 'X' mosaics, 18% 46,X iso(Xq) and mosaics 5% X/XY mosaics and 5% 46,XX/45,X mosaics.

Seven cases with 45,X chromosomal complements 3,X/XX to one with three cell lines X/XX/XXX were reported by Pauri in 1979 in his study on 11 cases of Turner's syndrome.

Out of three patients of 45,X, two had hypoplastic uterus and ovaries were not seen, while one had ovary on one side, the other ovary and uterus were not found. Similar findings were reported in Turner's syndrome to have normal or hypoplastic ovaries by Ullrich (1930; 1949) and Ferguson-Smith (1965). The other somatic anomalies found in the present study, anomalies were observed by Smith (1965), were shield chest, short neck and cubitus valgus.

In the present study, secondary sexual characters were found to be underdeveloped or absent. There is always certain amount of body hair. The axillary hair may be absent or very scanty; the amount of pubic hair is usually less than normal but more developed than the axillary hair.

The external genitalia in the cases of the present study are invariably infantile. The vulva has a rather flat surface. The labia majora are flat, while the labia minora remain thin and underdeveloped. The clitoris is very small. However, in a few cases, variation may be noted in the form of moderate clitoral hypertrophy and normal growth of pubic hair (Pich, 1937; Gorden et al, 1955; Grambach et al, 1955). The source of androgen in these cases may be in the gonads (hilus cell hyperplasia), in the adrenal gland or in aberrant adrenal tissue.

A variety of chromosomal errors may result in to 45,X chromosomal constitution. It may be a consequence of non-disjunction or chromosome loss during gametogenesis in either parent, resulting in lack of a sex chromosome in a sperm or in an ovum. Although the errors of mitosis in a normal zygote often lead to mosaicism, a purely 45,X constitution may arise at the first cleavage division from anaphase lag with loss of a sex chromosome. Loss of one X and a Y chromosome between fertilization and first cleavage division may be frequent, but is not the only cause of a 45,X embryo (Morishima et al, 1968).

X/XX Mosaicism

This is the second in frequency to 45,X and is the most common finding. In the present study, 6 patients were found to have 45,X/46,XX cell lines. Five of them had a main complain of primary amenorrhoea and short stature. One patient had a history of repeated fetal wastage. Patients with this form of mosaicism may have a clinical picture ranging from that of a complete Turner's syndrome to that of normal female phenotype, as found in the present study and so also reported by Grouchy et al (1964) and Smith (1965). It is also presumed that XX cell line has a diluting effect on the Turner phenotype and that the

proportion and distribution of XO cells are the deciding factors in the degree of variation (Ferguson-Smith, 1968). They are not invariably short and may menstruate and even be fertile (Grambach et al, 1985). Similarly, one case was found in the present study with repeated fetal loss.

From the data of 46 cases (Court Brown et al, 1964; de Grauchy et al, 1961; de la Chapelle, 1962; Ferguson-Smith et al, 1964; Ferrier et al, 1961; Hadded, 1962; Fraccaro et al, 1960; Lindsten, 1963; Mikkelson et al, 1963; Turner, 1964; Van Wijek et al, 1964; Warren et al, 1964) were summarized by Leao et al (1966) and Morishima et al (1968). In this X/XX group, cardinal features of Turner's syndrome were much less frequent that 45,X patients. Out of 46 patients, 7 had normal height and menstruation was not too rare (Ferguson-Smith, 1965). Less than 20% of cases were reported to have webbed neck and lymphoedema is in 45,X subjects. The X/XX mosaics were found to have mandibular hypoplasia, high arched palate, shield chest, cubitus valgus, low implantation of hair behind the neck, pigmented nevi and short fingers and toes. One had webbed neck, coarctation of the aorta, and a variety of other stigmata but was of normal stature and menstruating regularly. Normal vagina and streak gonads have been found in most cases subjected to the pelvic exploration. 80% cases in the present study were found to have streak gonads and hypoplastic uterus. Many women with X/XX escaped detection as it is quite possible that they have a normal ovarian function; only those with primary amenorrhoea or repeated fetal loss had been selected for the cytogenetic study. In most of the cases, they have been chromatin-positive (buccal and vaginal smears), but the percentage of positive cells was below normal range of that for normal females. In the present study also, percentage of X-chromatin-positive cells (4-10%) was found.

It has been suggested that X/XX mosaicism could arise in a 45,X zygote by mitotic non-disjunction in one of the 45,X cells any time after the cleavage division (Kobylinsky, 1883). An alternative mode of formation

could be loss of an X-chromosome in a normal XX zygote by anaphase lagging at the first or a later cleavage division.

It is well known that one X-chromosome undergoes heterochromatization in every part of the body of a normal female except the germ cells (Ohno et al, 1960). This suggests that the proper migration of germ cells from the hind gut endoderm to the primitive gonadal ridge requires two active X chromosomes. If one of them is lost or part of it is deleted migration does not take place and a streak gonad results. This may be true in 5 of the 6 patients in the present series where gonads were not seen. In the occasional case, where an XX sex chromosome complement is found in association with gonadal dysgenesis, there may be undetected mosaicism or a small deletion of the short arm of the X-chromosome. It is also possible that germ cell migration may be interferred with by mechanical, viral or other factors.

The development of the genital duct and external genitalia in gonadal dysgenesis always follows feminine lines, whatever the chromosomal constitution may be. This is in agreement with Jost's experiments (1947) on the castration of rabbit embryos prior to differentiation of the genital tract. In the present cases of 46,XX/45,X gonads were not seen but all the six patients had vagina and 4 had hypoplastic uterus, while one had normal ovaries, uterus and vagina.

Fourth patient of the present series had a consecutive first trimester abortions with 46,XX/45,X. This may be because of abnormal development of follicle. Abnormal folliculogenesis might be resulted in abnormal corpus luteum function, which is associated with repetitive pregnancy loss (Holzgreve et al, 1984). It might also be the hormonal milliue just after the LH surge or an abnormal chromosomal complement that can cause miscarriage.

Criteria for detection of mosaicism

Many investigators believed that one has to cultured and karyotyped multiple tissues, otherwise many cases of sex chromosome mosaicism will

be overlooked (Jacobs et al, 1961; Blank, 1964; Court Brown et al, 1964).

In order to test the validity of the assumption that the readily available white blood cells often do not reflect the chromosomal situation in other tissues; Ferrier et al (1970) carried out studies, and contradictory results obtained. They studied 31 cases of Turner's syndrome, 8 cases of male pseudohermaphroditism and 4 cases of Klinefelter's syndrome by culturing cells from other tissues, as well as from peripheral blood.

Only in one case of Turner's syndrome, there was mosaicism in skin fibroblasts, which was not detected in leukocyte cultures. In one of the pseudohermaphrodite patient, mosaicism was present in leukocytes only. All patients identified in this study as mosaics had at least 5% the total number of cells in the minority cell line. It was concluded that very few instances of sex chromosome mosaicism would escape detection if leukocytes culture alone were carried out.

The patients of de la Chapelle and most patients of Engel and Forbes (1965) were studied by leukocyte cultures only, whereas, the majority of patients investigated by the other workers were studied by cultures from multiple tissues. The proportion of mosaic patients is roughly the same in all series, indicating that leukocytes cultures are adequate for demonstrating mosaicism (Ferrier et al, 1970).

There was some interesting results found in skin culture. The skin culture takes relatively long time in culture (compared to lymphocyte culture) allows time for random fluctuation or even cell division in vitro. It is likely that small skin biopsy may not be representative and the cells which grow out and establish the culture may not be representative of the biopsy. Fluctuations in results of mosaicism were found in successive skin biopsies which reinforce the low reliability of skin culture in mosaics. Analysis of gonadal tissues would theoretically detect additional cases and help in understanding the mechanism of expression of the sexual phenotype but in practice relatively few examples are known in which gonadal fibroblasts were 45,X/46,XX and lymphocytes 46,XX (Simpson,

1975; Simpson et al, 1971). Lejeune et al (1966) reported a case of XX/XY chimerism in which both the XX stem line was detected in a testis culture. Analysis of germ cells might detect additional monosomic cells, but in gonadal dysgenesis this is, by definition, impossible. As a general rule, both stem lines are most often found in blood cultures (Lejeune et al, 1966). Another consideration is mosaicism is the possibility that the sexual phenotype will depend on the relative proportion of the stem lines in the premature gonads (Jacobs et al, 1961). In the present study because of the unavailability of other tissues like skin, gonads for culture, chromosome studies were carried out only from whole blood culture.

In the mosaic the number of cells that need to be screened will depend upon the percentage of the minor cell line, which in turn probably depends upon the time mosaicism originates. Ford (1969) calculated the number of cells necessary to exclude statistically minor cell populations. The probability that a random sample of cells from a mosaic subject with two cell lines will not include at least one cell of the minor cell line that is present in a given frequency is expressed by $(1-p)^n$ where p is the frequency of cell line in question and 'n' the number of cells in the sample for sample of 30 and 50 cells and for a frequency of 0.05 the probabilities are 0.214 and 0.077 respectively, if the cell line comprises only 5% of the cells in the population, it would pass unsuspected approximately once in 5 trials if the sample was increased to 50 cells. If the routine examination arouse suspicion of a mosaic condition, the number of metaphases examined was increased until, eventually, the number of recorded cells from the minor cell line was higher than could reasonably be attributed to chance. Kuleshav et al (1976) presented a method for diagnosis of mosaicism in patients studied for clinical reasons and in subjects investigated a population studies, giving confidence limits of probabilities for various degrees of mosaicism. In clinical cytogenetic studies the initial analysis was to be performed on 29 cells. If these 29 cells were of the same chromosome constitution, then there was a strong possibilities (with 0.95 probability) that there was no mosaicism at 10% level or more. Alternatively, if one cell with another chromosome

constitution was found in these 29 cells, then the analysis was intended to more cells. Over and above in the patients with mosaicism culture were repeated after 3 months to 1 year period in the present study.

46,X, iso(Xq) - X-isochromosome

In the present study, there are two patients with 46,X iso(Xq). As the large number of cytogenetic syndromes have proliferated, the correlations of karyotype and phenotype have become increasingly important to enable clinician to utilize available clinical clues as aids in differential diagnosis. This is exemplified in classical 45,X Turner's syndrome, which must be separated out from numerous other syndromes involving structural abnormalities of the X-chromosome. X-isochromosome. X syndrome is one of the most confusing abnormalities, where the patient presents with some, or many of the features of 45,X Turner's syndrome, yet has a positive buccal smear for the X-chromatin body. The mis-division of a centromere may lead to a formation of an unstable telocentric isochromosome (Darlington, 1939). Fraccaro et al (1960) were the first to describe Turner's syndrome with an isochromosome for the human X.

Both the patients with iso(Xq) in the present study show short stature, absence of secondary sexual characters. Absence of menstruation, cubitus valgus. One patient with streak gonads, hypoplastic uterus and normal vagina. While in other patient, under ultrasonography examination gonads were not found, uterus was not palpable as well, but length of vagina was normal. Many patients with 46,X iso(Xq) like 45,X Turner were reported to have short stature, streak gonads, lack of ovarian follicles, amenorrhoea, short-IV metacarpals and pigmented nevi, by Ferguson-Smith (1965); Faccaro et al (1960); Engel et al (1961); Klotz et al (1962); Hamerton et al (1962); Lindsten (1963); Spars et al (1963); Court Brown et al (1964); Morishima et al (1968); Mattevi et al (1971); Otto et al (1981). Some of the features of Turner's syndrome like short neck, hypoplastic nails, lymphoedema, congenital cardiovascular defects and high arched palate are less frequently seen. It is interesting to note that there is an unusual frequency of thyroiditis in the 46,X iso(Xq) syndrome. Santana et al (1977) observed mental retardation, partially developed

nipples, thyroiditis to be higher in cases of isochromosome. Both the present cases found to have all these findings. An association between thyroid autoimmunity and the 46,X iso(Xq) have been reported by many investigators (Bahner et al, 1960; Grumbach et al, 1964; Williams et al, 1964; Ferguson-Smith, 1965; Grumbach et al, 1964). The exact role of the isochromosome in the pathogenesis of the Hashimoto's thyroiditis still remains unknown.

Many investigators (de la Chapelle et al, 1974; Yanagesawa, 1973; Ruthner et al, 1974a) reported different morphologies in the patients with isochromosome or duplication of the long arm of the human chromosome, which affects the phenotypes differently (Gall et al, 1981; Laszlo et al, 1977). Duplication of X-long and short arms are now confirmed by banding studies (Darlington, 1939; Diesteche et al, 1972; Berghe et al, 1973; Ruthner et al, 1974b; Therman, 1974b). According to Schmid et al (1974), mosaics frequently occur because this dicentric chromosome is unstable and can disappear from the cells. Fujita et al (1977) suggested that the dicentric chromosome is not always unstable as they found C bands on the dicentric chromosome was always biparticle in each chromatin and lost its centromeric function. Morphological differences in isochromosomes for the long arm of the human chromosome are interesting both for delineating the phenotype associated with abnormal X-chromosome and for the understanding of isochromosome formation because iso(Xq)s are generally inactivated and may also be associated with mosaicism (Muldal et al, 1963). In such case, the maintenance of fertility is disturbed which may be due to the absence of one short arm, that might carry a gonadal determinant. The genes present on the long and short arms of both X-chromosomes are necessary for the development of a functional ovary (Ferguson-Smith, 1965; Jacobs, 1969), because in 46,XXP and 46,XXq females, the normal ovaries are replaced by streak gonads. The same streak ovaries and absence of uterus were found in the present study. In females with 46,XXPC or 46,XXq complement absence of both short stature and other somatic abnormalities associated with 46,XX complement, were absent. Furthermore, they suggested that genes apparently influential on

the development of stature and of the other somatic features which are frequently abnormal in females with a 45,X complement, are situated on the short arm of the X-chromosome.

Autoradiography studies have shown that the isochromosome is the late replicating one (Muldal et al, 1963; Geanelli, 1963; Atkinz et al, 1964). Late-replicating structurally abnormal chromosomes give rise to the X-chromatin in body. Now it is proved that the gene located near the centromere in proximal part of long arm is associated with the formation of the Barr body. In iso(Xq) condition the gene responsible for the formation of Barr body is in double dose. Hence, X-chromatin bodies are larger than normal in patient with a 46,X iso(Xq) condition. Late-replicating study was also done in the present case of iso Xq and found the same result.

45,X/46,X + Marker Mosaic

The patient with 45,X/46,X + marker, observed to have Turnerian features in the present study. She had a primary amenorrhoea, short stature, underdeveloped secondary sexual characters, absence of axillary and pubic hair, low hair line, cubitus valgus, shield chest, increase distance between big toe and second toe.

60% of the cells found to have 45,X; whereas, marker cell line was found in 40% of cells. Four patients with 45,X/46,X + marker chromosome were reported by Gemmill et al (1987), referred for suspected Turner's syndrome. In all patients, they found short stature, primary amenorrhoea, cubitus valgus, low hair line, absence of secondary sexual characters with shield chest. The same findings were observed in our patients. Gemmill (1987) found two acrocentrics, one small ring and other had similar appearance of the Y-chromosome with deletion of Yq 12 - marker chromosome. In the present study, marker was metacentric chromosome. G-banding was not very clear. Gemmill et al (1987) suggested DNA hybridization and southern blot analysis for further confirmation as he found Y-specific DNA sequences. They further recommended both molecular

and cytogenetic analysis in the patient with suspected Y-chromosomal material, in a phenotypic female.

Testicular Feminising Syndrome (TFS)

In the present study, TFS was present in 3 phenotypic females. Mental and physical growth were found normal. Overall height was less than span. One patient (KL) had height 160 cms/span 164 cms. She had enlarged clitoris, blind vaginal pouch, left testis was in labia and the right one in inguinal canal.

Second patient (RB) had normal external genitalia with blind vaginal pouch, well developed breast. Testes were inguinal. There is absence of uterus and ovaries. Third patient was a small baby. Testes were found in labia majora. External genitalia were female type, with vaginal opening.

Testicular feminization syndrome (TFS) is very likely diagnosis when a blind vaginal canal is found and uterus is absent. In the patient of TFS, individuals will have 46,XY, bilateral testes, external genitalia female type, no Mullerian derivatives with the vagina ending blindly that means genitalia are opposite to the gonads, hence such patient is a male pseudohermaphrodite. According to Simpson (1978, Review) affected individuals show breast development, like normal female, clitoral enlargement and labioscrotal fusion. The male pseudohermaphrodite is a genetic and gonadal male with failure of verilization.

It is now evident that the embryonic testis is competent only in suppressing Mullerian duct development, but if fails to stimulate Wolffian duct development or to masculinize the external genitalia. Jost's experiments (1947) on the effect of castration of the rabbit male fetus in utero showed that masculinization of the external genitalia and Wolffian ducts is androgen-dependent. The lack of this masculinizing effect in the present study cases may be explained by either insufficient androgenic secretion from the embryonic testis or "biological non-activity" of normal amounts of these androgens. It seems that in this syndrome, latter is the more probable explanation as the testicular androgen fails to virilize

these patients at puberty inspite of their production in large amount in the present cases. Similar observations were reported by Griffiths et al (1963); Morris et al (1963) and Kase et al (1965). It has also been found that the administration of large amounts of steroidal androgens fails to masculinize these patients. These points seem to be in favour of Wilkins (1957) and Prader's (1957) suggestion that the testes in this condition are producing sufficient androgens but the defect lies in the end-organ response. Thus, fetal development can be explained by the non-action of androgen and the presence of testicular Mullerian inhibiting substance, while the development of feminise secondary sex characteristics at puberty is explained by the non-action of androgens plus the testicular estrogens. Failure in male development can be considered as a spectrum with incomplete forms of testicular feminization being represented by some androgen response. Transmission of this disorder is by means of an X-linked recessive gene or a male limited autosomal dominant that is responsible for the androgen intracellular receptor (Speroff et al, 1984). Looking to above observation, clinical diagnosis of TFS would be considered in patient :

1. a female child with inguinal hernias, because it is likely that testes are frequently partially descended,
2. patient with absent body hair
3. patient with primary amenorrhoea and an absent uterus.

Stegleher (1817) and Ricco (1932) have reported complete testicular feminization in the individuals. Several reports by numbers of investigators were available in early part of this century. After the tabulation of 98 patients by Morris (1953), the TF was considered as distinct entity. TF appears normal at birth; except for the possible presence of a hernia found in two patients and both of them have not seek advice of physician till puberty. Presence of an inguinal hernia in phenotypic female is the only feature which claims the TFS. Occasionally TF patients are identified by only cytogenetic studies. Jagiello et al (1962) and German et al (1973) reported few patients without inguinal

hernia. Assuming one case of TF per 120 phenotypic females with inguinal hernias, Jagiello et al (1962) estimated incidence of the triat to be 1:62,400 males.

One case in this study with TFS was quite attractive and had excellent breast development. She showed female psychosexual behaviour. Same characters were reported by Money et al (1968) and Simspon (1978 - Review Article).

External genitalia are undifferentiated, more of female typed in two cases but indistinguishable from those of normal females. In one case, clitoris is larger than usual. In complete form of TF the clitoris is not found to be so large that the sex of rearing is questioned. There are rudimentary fallopian tubes with fibromuscular tissue with only occasional epithelial call lining. The vagina is shorter than usual and blind in two cases, presumably because of undifferentiation of Mullerian derivatives which could not contribute to the formation of the vagina.

Anatomical and embryological findings suggest that the testis may be located along the path testicular descent i.e. in the abdomen, inguinal canal, labia, in the TF patients. In three cases of the present study, testes were found in inguinal canal. Histologically the testes of prepubertal patients are similar to the undescended testis of normal males, but in the post-pubertal testis found to have (this correlates with the biopsy of one patient in the present study) :

1. under-developed seminiferous tissue with sartoli cells,
2. few cells : spermatogenic and no spermatozoa,
3. Leydig cells hyperplasia and found in clusters.

Many investigators reported increased frequency of gonadal neoplasm in complete testicular feminization. They also believed that the risk of neoplasia below 25-30 years is relatively less; although the etiology of the neoplasia remains unknown (Hanser, 1963; Jones et al, 1971; Morris et al, 1963; Simpson et al, 1976; Manuel et al, 1976; Teter, 1976). Due to

the relatively high incidence of malignancy, it is wise to remove these gonads after development of the secondary sexual characteristics and then to maintain the patient on estrogen substitution therapy. The 17-ketosteroid urinary excretion is normal and elevated urinary estrogens show normal male or female values and cornification is noted in the vaginal mucosa. Pituitary gonadotrophins are normal or slightly elevated. Following castration the 17-ketosteroids and estrogen levels fall, vaginal cornification diminishes and gonadotrophin levels rise.

The levels of testosterone, dihydroepiandrosterone sulphate and androsterone sulphate in the peripheral blood are within normal male values (Pion et al, 1965). Same was found in one patient of the present series. According to Bardin et al (1973) due to the cytosal androgen receptors in the target organ, testosterone in plasma do not respond to androgens either their own or those given locally or systemically. Therefore, the critical steps in sexual differentiation which require androgens fail to take place and development is totally female type. This may be the reason for all the three patients studied.

Klinefelter's Syndrome

In the present study, 13 patients were found to have Klinefelter's syndrome. Out of 13, the patients with 47,XXY were 7; with 46,XY/47,XXY were 3; double trisomy 48,XXY +21 one and 46,XXY +15 one. At the adolescence patients came with the complaint of gynaecomastia, in 5 patients absence of secondary sexual characters, small under-developed phallus with small atropic testes with hyalinization of the seminiferous tubules; aggregation of Leydig cells, aspermatogenesis and increased urinary excretion of gonadotrophins were the findings. Similar to the findings reported by Froland (1969), Hsueh et al (1978), Leonard et al (1978). Sperms were reported to be present in seminal fluid of at least 6 cases (Ferguson-Smith et al, 1957; Fatterweit, 1967; Raboch, 1957, 1964). While all the 13 cases were azoospermic in the present study. Pasqualini et al (1957) and Mosui et al (1960) reported high incidence of mental deficiency in association with the Klinefelter's syndrome, though in the present series, 3 were found to be mentally subnormal. According to

Rhode (1963) about ½ of all chromatin-positive (XXY) males with this syndrome are mentally deficient. In the present study, one patient with 47,XXY and one with 46,XXY +21 other with 48,XXY +15 were found. They were referred to rule out the cause of mental deficiency and azoospermia.

There is an increased incidence of problems with speech development, barring at school and social adjustment in adolescence were reported by patients in seven cases. With increasing age, one finds an increased expression of the 'endocrine psychosyndromic' of Bleuler (1955), impulsive excitation and defection states, diminished heterosexual interest, increasing impotence and general loss of initiative, antisocial behaviour and deliquency, also reported from retrospective studies, although the exact risk is uncertain (Polani, 1981).

Klinefelter's syndrome commonly recognized at puberty. 60% patients in the present series came at puberty with the complains of infertility. Other seek medical advice for gynaecomastia or small testes. Few may come with the complains of erections and intercourse problems. In 60% of the patients the disproportionate length of the legs were found. The same were reported by Stewart et al (1982); Polani (1981), Scheller et al (1974) - patients become taller than average.

Meiotic non-disjunction of the sex chromosomes during either the first meiotic division in either parent seems to be the basis of the abnormal sex chromosomal findings in this syndrome. Less commonly meiotic non-disjunction in the zygote at the time of or following fertilization was reported. Increased maternal age and parental irrediation have been suggested as relevant factors in etiology of this condition (Ferguson-Smith, 1963). Though these factors could not be recorded as patients do not remembered. Rosenkranz (1965) has reported 47,XXY patients whose mothers were abnormal; one was 47, XXX and the other was 46,XX/47,XXX mosaic.

In the present study, the hormonal findings were, increased gonadotrophin excretion in urine, 17-ketosteroids are mostly normal or slightly

decreased. There is a deficiency of androgens at all ages. Massive doses of chorionic gonadotrophin increases, 17-ketosteroids and/or the estrogens may show a transitory increase, as also observed by Leon et al (1959); Seringe et al (1961). This indicates that some functional activity of the Leydig cells is still present. This was confirmed by histopathology which showed numerous crowding growth of Leydig cells.

XX/XY Mosaicism

The condition may be found because of errors during mitotic cleavage in early division of normal XY zygote or random fusion of 46,XX and 46,XY blastocytes seldom produces 46,XX/46,XY true hermaphroditism (Jarkowshi, 1961) (Fig.). Out of the three cases found to have XX/XY mosaicism, one 31 years old female referred for spontaneous abortions of anembryonic pregnancies. Phenotypically, she was a normal female. Her cytogenetic finding was 46,XX/46,XY. Many investigators like Tiltman et al (1982); Williamson et al (1981); Kim et al (1979) have reported 14 pregnancies in true hermaphrodite reared as female, resulting in 12 live offsprings. Williamson et al (1981) and Kim et al (1979) suggested that patients should be modulated as the uterus is sometimes abnormal with stenosis of the cervix or the fallopian tube. It was not possible to do gonadal biopsies because of patient refusal.

Second case was 10 years boy with hypospadias. He had male type external genitalia, penis normal in size, bifid scrotum with rugosity, but the testes were not palpable. Patient did not come for follow up and hence gonadal biopsies could not be done. Last case was 17 years old female referred for ambiguous genitalia. Her cytogenetic findings were 46,XX/46,XY. She had ovo-testis on right side and functional ovary on the left side. Many investigators like de Merchi et al (1976); Nihoul Fekete et al (1984); Niekerk (1976); Niekerk et al (1981) have reported similar cases in the past literature.

True Hermaphroditism

In the present study, out of 3 cases of XX/XY mosaicism, one case from histopathology examination of biopsy masses revealed ovotestis on right side and ovary on left side.

According to Jones et al (1971) and Van Niekerk (1976; 1981) true hermaphroditism requires the presence of both ovarian and testicular tissue in either the same or opposite gonads. Van Niekerk et al (1981) reported 29.11% of cases with an ovotestis on the one side and an ovary on the other side - unilateral true hermaphroditism. They also observed ovotestis on right side in 57.7% and on the left side in 42.3% in the study of 409 cases of true hermaphroditism. Ovary was found on the left side in 62.8% and on the right side in 37.2%. This review indicates that ovary occurs most commonly on left side while ovotestis or testis has a greater tendency to occur on the right side of the body.

Most of the cases were reported to have ambiguous genitalia, with few with either male or female type. Because of the size of the phallus the patients are reared as males. Almost all have hypospadias which vary in extent from perineal to penile, with complete fusion of labioscrotal fold. In the present study, a 17-year old girl with ambiguous genitalia reared as a male upto the age of 13, because of the size of the phallus and incomplete fusion of labioscrotal fold. She started menstruating at regular intervals at the age of 13. Her cytogenetic examination showed 46,XX/46,XY.

Inguinal hernia containing gonad or uterus is reported in about one-half cases of this type. Differentiation of the genital ducts usually follow that of the gonads. The ovotestis is the most common gonad found in true hermaphrodites, followed by ovary and, least commonly the testis. The patient with ovotestis has predominantly female development of the genital ducts. The relationship between gonadal structure and differentiation of the genital tract in true hermaphroditism provides added evidence for the local effect of the Mullerian inhibiting substance secreted by the fetal testis. In the present case, uterus was not seen.

In the present study, testicular biopsies taken showed less evidence of tubular maturation, and no increase in the amount of interstitial fibrous tissue; these changes were similar to those found in simple cryptorchidism. Catelli et al (1980) and Aaroson (1985) reported similar

changes in the germ cells found in cryptorchidism. The reason, however, for the degeneration of testicular tissue in true hermaphrodities remains a matter of speculation (Donachoe et al, 1978).

Presence of Y-chromosome in 46,XX/46,XY true hermaphrodites resulted in to the development of testicular tissue of ovotestis. In some of the cases with 46,XX true hermaphrodites they developed ovotestis in absence of Y-chromosome. In such cases, there may be a possibility of hidden Y-containing cell lines. Some investigators (Grambach et al, 1985) reported that 46,XX true hermaphroditism may result from translocation of testicular determining gene(s) on the Y-chromosome to either an X-chromosome or an autosome or from an autosomal recessive or dominant mutant gene. However, Wachtel et al (1979); Clayton et al (1958); Mori et al (1968) observed some familial forms of XX true hermaphroditism are consistent with autosomal recessive inheritance and compatible with autosomal transmission (Fraccaro et al, 1979). Though such translocation could not be observed in the present study.

Spontaneous Abortions

Seventy couples were cytogenetically studied to rule out the genetic cause for repeated fetal wastage. In three females, numerical 45,X/46,XX; 46,XX/46,XY and structural abnormalities in form of balanced Robertsonian translocation 45,XX-13, -14 t(13q/14q) were found. First two cases were discussed in their respective group.

Abortion is one of the commonest complication of pregnancy. The factors lead to early abortions are often unknown but it is associated with uterine abnormalities like bicornuate uterus, hormonal disturbances, infectious diseases, and immunological factors (Glass et al, 1978). According to Holzgrene et al (1984) nearly 50 to 60% of all first trimester abortions are associated with some numerical and structural abnormalities of chromosomes. Cour Brown (1967) reported Robertsonian translocation (RT) balanced or imbalanced is one of the common structural abortions found as one of the cause in early pregnancy loss. Incidence in live born infants varies from 1-2 per 1000 (Jacobs, 1977; Hamerton, 1982). McDonough et al

(1981) observed increase risk of recurrence with RT. In the present study, 25 year old female phenotypically normal with normal sex chromosome to have RT.

Review of past literature showed that the balanced chromosomal rearrangements in a parent may predispose to recurrent fetal wastage (de la Chapelle et al, 1973; Khudra, 1974; Papp et al, 1974; Tho et al, 1979; Husstein et al, 1982). 4-14% of recurrent aborted couples found to be a carrier of a cytogenetic abnormality, with or without fetal malformations. Such chromosomal aberrations found to occur during gamatogenesis resulted into unbalanced gamate formation, giving rise to maldevelopment of early abortion. It may also give rise to a balanced gamate resulting in a balanced carrier or it may produce cytogenetically normal as found in the present study.

Ambiguous Genitalia

Human sex determination and differentiation are sequential process. The chromosomal (genetic) sex is determined in the zygote at the time of conception. The gonadal sex is determined in response to genetic sex. This gonadal sex regulates the differentiation of the genital organs that ultimately defines the phenotypic sex. At puberty, the development of the sex specific secondary sex characteristics, reinforce and provide more visible phenotypic manifestation of this sexual dimorphism. Ambiguity of external genitalia may result from an abnormality in genetic sex (sex chromosomal abnormalities), gonadal sex (ovotestis or streak gonads) and phenotypic sex (male or female pseudohermaphroditism) (Rache et al, 1976). Differentiation of primary gonads into a testis is determined by the Y-chromosome and the testicular determining gene present on sex chromosome autosomes. Differentiation, nor development and masculinization of the genitalia and the descent of the testis, are governed by hormones, which include sex steroid testosterone and Mullerian-inhibiting substance secreted by fetal testis. Further conversion of testosterone to dihydrotestosterone by external genital tissue in the presence of enzyme 5-reductase. Dihydrotestosterone combines with androgenic specific tissue receptors to produce virilization of the external genitalia.

The male and female embryo both possess undifferentiated common gonadal primordia that has an inherent tendency to feminize unless there is active interpresence by masculinizing factors. Moreover, female differentiation of the somatic sex structure (the internal and external genital tract) occurs independently of gonadal hormones and which emerge in the absence of fetal testis whether ovaries are present or not. Thus, the sexual dimorphism in phenotype that results from differentiation is mediated by the fetal testis and its dual hormonal secretions and not by the ovary. Male differentiation in the presence of testis takes place despite an environment in which the concentration of circulating oestrogens and progesterones is high. Defect at any level can cause ambiguity of the genitalia. The hypospadias is generally found to be associated with genital anomalies like microphallus with severe chordae, penoscrotal transposition, bifid scrotum, small gonads and/or cryptorchidism in male. In female, hypertrophied clitoris, absence of vagina, labio-scrotal fusion, inguinal swelling etc. are found. Most of the cases of the present study have been discussed. The remaining showed slight variation in phenotypic features correlated with cytogenetic sex.

In the individual with ambiguous genitalia proper assignment of sex for rearing and appropriate subsequent management, help the individual to lead well adjusted lives and also to attain a satisfactory sex life. In some cases, clinically assignment of sex of rearing is difficult in such cases cytogenetic sex is inevitable. After knowing the genotypic sex, surgical corrections can be made to assign the proper sex. Once the sex is assigned, ambiguity can be corrected surgically, by hormonal treatment ultimately psychological measures.

XY-agonadism

Once the differentiation of primordial gonad into testis occurs, primary action of fetal testis is to synthesize and secretes a peptide like factor in the seminiferous tubules - sertoli cells - which induce the Mullerian duct regression (Jirasek, 1976). Leydig cells of fetal testis secrete fetal androgens which induce virilization of both the Wolffian derivatives and the genital tubercle (Jost et al, 1973). The regression of fetal testis, may

be due to unknown cause, result into an XY-agonadal individuals. In such individuals phenotypic features may vary from complete female to male phenotype with genital ambiguity.

The patient 20 years old male, in the present study, showed male phenotype, XY-chromosome constitution and elevated gonadotrophins with very low levels of serum testosterone. This may be due to :

i) failure of Mullerian duct regression,
ii) late testicular regression resulting in an unusual association of rudimentary uterus and bilateral anorchia.

This explains the virilization observed in the case under report. Krischer et al (1970) and Parks et al (1974) have documented the presence of testosterone secreting tissue even in the absence of testis. The present case is a probably variant of the gonadal dysgenesis with absence of gonads. The endocrine and histopathological studies fail to demonstrate the presence of functional of non-functional gonads. Persistently elevated levels of serum FSH and LH with extremely low testosterone levels suggest normal pituitary function and absence or unresponsive gonads. Absence of significant rise in serum T following HCG stimulation confirms the absence of functioning testicular tissue. The endocrinological findings of the present case are also similar to those of reported in agonadal individuals or to functionally prepubertal castrated males by Kofman-Alfaro et al (1976); Root et al (1972); Yen et al (1972). The present investigation, therefore, suggests the occurence of one of the following conditions :

i) Testicular or gonadal dysgenesis or agenesis,
ii) Leydig cell irresponsiveness to HCG stimulation.

During embryogenesis, if testicular regression occurs prior to male sexual differentiation a female phenotype should develop. However, in the present case, the absence of gonadal tissue and lack of T even after HCG stimulation are strongly suggestive of testicular regression after completion of male sexual differentiation.

In other reported patients, the three sons born to a non-consanguinous parents were studied in the present study. They were found to have a new variant of 46,XY gonadal absence syndrome. In all the three cases, S.FSH and S.LH persistently low and with extremely low testosterone levels suggest hypogonadotrophic hypogonadism. A similar case was reported by Gregoric-Polacios et al (1978). In these three cases, it is likely that the embryonic testis regression occurs later on but before completion of male sexual differentiation the final result will be ambiguious genitalia as also reported by Bergada et al (1962); Najjar et al (1974). Involvement of three brothers in the same family with similar genotypic and phenotypic findings suggest possible mutant gene effect.

Aleyaratne et al (1969) and Kolodney et al (1971) referred to such a condition as a "vanishing testis" syndrome. This condition is characterised by development of Wolffian system with concomitant absence of Mullerian derivatives. Similar findings have been reported by Jasso et al (1980) in two siblings with microphallus and anorchia. The co-existence of 46,XY gonadal agenesis and anorchia in the same siblings suggests that both the disorders are related and that "testicular regression" occured at different stages of male development, this also suggests that there may be operation of a rare mutant gene in some cases of this syndrome.

The case (FR) had right descended testis with right side inguinoscrotal hernia. Opening of hernial sac showed uterus with fallopian tube with testis. Histopathological examination of uterus revealed endometrium and myometrium. Microscopic examination showed testicular atrophy while right descended testes show functioning testes. Karyotype 46,XY and abnormal presence of uterus suggestive of dysgenetic male pseudohermaphordite. The presence of uterus suggests that there is an either failure of Mullerian duct inhibitory factor (MIF) secretion or an altered MIF, molecule or there may be a target organ resistant to MIF in this case as has been suggested also by Brook et al (1973).

47,XXX - Female

A number of triple X phenotypic females have been reported to be afflicted with some other clinical findings (Barr et al, 1969; Hamerton,

1971; Smith et al, 1974; Mueller-Henbach et al 1977; Chandley, 1979) after the first description by Jacobs et al (1959) of the 47,XXX normal female phenotype patient with secondary amenorrhoea. The incidence of trisomy X is approximately 1/1000 live born females (Hamerton et al, 1975). The association between the XXX abnormality, clinical features and mental development is well known (Smith et al, 1974). The etiology of trisomy X is uncertain, it may arise by different mechanism, but the exact origin of parental non-disjunction could be identified in the present cases.

The height of the patient suggests that there may be a growth factor favouring tall stature as well as hormonal imbalance, associated with increase in X-chromosome number. This confirmed the earlier observations of Collen et al (1980) that increased dose of X results in height and hormonal disturbances.

Smith et al (1974) reported premature ovarian failure in 13 cases with 47,XXX karyotype. They found in one case of primary amenorrhoea, cystic ovaries on both sides. While in other right ovary was cystic and the left one was absent. In one case (SM) of the present study, there were both ovaries but absence of uterus. In the second case, left ovary was absent and right ovary was underdeveloped. Third case with 46,XX/47,XXX found to have short stature, underdeveloped secondary sexual characters including breast, there is absence of both ovaries but presence of uterus. Her short stature explained the dominating 46,XX cell line diluting the effect of 47,XXX cell line. The last case (SB) with secondary amenorrhoea had a history of a commencement of menstrual cycle at the age of 15 years. Later on menstrual cycle became irregular and discontinued. Similar condition of secondary amenorrhoea was reported by Smith (1974) in two patients having history of a single menstrual period. Ovaries were found to be small in one patient and fibrous in the other. Present case showed 46,XX/47,XXX. Sharma (1985) has also described similar cases of triple X females with oligomenorrhoea and/or premature menopause. Mental retardation was also common in these cases. In our study, however, no patients were found to have any indication of mental retardation.

As per the present observation and reports from past literature, a single X may be sufficient for early ovarian differentiation, two X-chromosomes are needed for normal ovarian development and function. The presence of an additional X-chromosome appears to diminish gonadal potential (Collen et al, 1980). In one case, the elevated levels of S.FSH and S.LH with low levels of E_2 and progesterone suggest the primary ovarian failure and this is correlated with laparoscopic reports which indicate presence of one underdeveloped ovary, and absence of ovary; and in other case, both ovaries were absent. Similar findings were reported by U.Radhakrishna et al (1991) in a triple X female with long arm deletion of one of the X-chromosomes associated with primary amenorrhoea : 47,XX t(X)(q 27-3). The abnormal X-chromosome in genome is always late replicating (Ruthner et al, 1979; Brankouce et al, 1979; U.Radhakrishna et al, 1991).

PART-III

In the present study, the endeavour was to first standardise the human chromosome methodology using all the locally available resources. The aim was also to maintain the best quality of chromosome preparation, so essential for the interpretation of the different types of observations.

Methodology

The first change in the methodology of the preparation was in the collecting media. The recommendation is the use of any nutrient medium like TC 199 (Difco, U.S.A.), Ham's hapten media or RPMI 1640 with fetal calf serum. The other recommendation is to add the patient's serum or AB serum to the media.

In the present study, bone marrow aspirates were incubated only for colchicine treatment and due to inherent high mitotic activity, cell proliferation was not the consideration. Keeping this in mind, use of expensive nutrient media was considered dispensable. To check the validity and reliability of this supposition in the initial stages of the present study, bone marrow aspirates were collected in two separate bottles containing lactated Ringer's solution in one and nutrient media TC 199 in the other and both were processed in the same manner. The results by both the techniques were compared; mainly the number of metaphases, the quality of chromosomes-specially, the length and clarity, chromosome spread and staining characteristics, chiefly the banding, were considered. It was observed that both the sets of collecting media yielded similar preparation and one with lactated Ringer's solution was not found to be inferior on any count. The advantages of using lactated Ringer's solution are its very low cost, simplicity in preparation of the final solution, no dispensing with costly impoted media and the results comparable with nutrient media. Similar method, without nutrient media, is also recommended by Tjio and Whang-Peng (1979). After these observations, lactated Ringer's solution was used throughout the present study as a culture medium.

Dry tap

The other problem faced specially in the patients with haematological malignancies is failure to get marrow particle or a dry tap. For cytogenetic analysis this might create problem specially where bone marrow culture is the method used, as in the present study.

In the cases where apparently no particles were seen and aspirate mainly contained blood (bloody tap) they were nevertheless processed because occasionally satisfactory preparations can be obtained from such specimen (Tjio and Whang-Peng, 1974). In the present study, 5 patients had bloody tap and the normal process yielded satisfactory results.

In the present study, the method for cytogenetic analysis in the patients with dry tap was to use bone marrow trephine biopsy piece. This technique yielded acceptable results.

In the patients with leukemia specially acute type, population of proliferating immature cells (chiefly blast cells) in the peripheral blood is quite high. In such cases, even peripheral blood culture without mitogen will also yield satisfactory results (Sandberg and Abe, 1980). In the present study, in 5 patients with dry tap and peripheral blast count of more than 30% of this type of short term blood culture was tried. Due to inexplicable reasons in none of the five cases acceptable results were achieved. In all the five patients, final cytogenetic analysis was done with bone marrow trephine biopsy.

So in the present study, bone marrow trephine biopsy was preferred to peripheral blood culture in those cases where bone marrow aspirate was a dry tap.

Slide preparation

The other observation in the present study was regarding the preparation of slides. For leucocyte culture, air-dried technique is considered the best, chiefly due to better spread and better results with banding as compared to flame dried method.

In the present study, flame-dried technique was used. In 20 cases, slides with both the methods were prepared and the results were almost identical as far as chromosomal spread was concerned. Staining by banding technique was not satisfactory by both the methods. de Grouchy and Turlesu (1978) have recommended use of flame-dried method over the air-dried when bone marrow culture is being processed. The reasons for the recommendation are not mentioned.

Banding technique

In the present study, G-banding was used for all the patients. It is reported by almost all the workers that the quality of chromosome in general and banding in particular is not good in the cases of haematological malignancies. The same problem was faced during the present study where throughout the quality of banding was not so satisfactory. Even the response of standard 120 minutes colchicine treatment varied from patient to patient with some chromosomes over contracted while others were too ill-defined, fuzzy and poorly spread.

In the present study, totally 40 consecutive patients with different haematological malignancies were studied as shown in Table-19 below :

Table-19 : Disease-wise distribution of 40 cases of haematological malignancies

Type of disease	No.of cases	Percentage of total 40 cases
CML	12	30.0
CLL	03	7.5
AML	04	10.0
ALL	03	7.5
HL	10	25.0
NHL	06	15.0
MM	02	5.0
Total	40	

All these patients were subjected to cytogenetic analysis and successful karyotyping was possible in 36 cases. In the remaining 4 cases karyotype was not possible. The disease-wise success rate of karyotyping is shown in Table-20 below :

Table-20 : Disease-wise success rate of karyotyping in 36 cases

Disease	CML	CLL	AML	ALL	HL	NHL	MM	Total
No.of cases	12	03	04	03	10	06	02	40
No.of successful karyotyping	12	03	04	02	07	06	02	36
Failure rate (%)	-	-	-	33.33	30.0	-	-	10.0

The failure rate in this series was 4 cases out of total 40, i.e. 10.0%.

No data regarding combined failure rate in all the haematological malignancies is available. But in one series of 159 patients of AML, failure to get metaphases was reported in 18 patients or 11% of total (Garson, 1980). In the same series, it was postulated that these patients constituted a special group which did not readily achieve remission and therefore, had a poor prognosis. Similar observation relating prognosis and non-responders has not been made by others.

In the present series, 2 patients (numbers 21 and 30) did not show any metaphases while numbers 24 and 33 did show few metaphases but were not at all spread. Of the first two cases, numbers 21 and 30; number 21 had ALL and his remission lasted for only two months and he was brought again in a bad state with recurrence. Case number 30 had presented with HL stage IVa. After three courses he has achieved partial remission and further follow up could not be done. In both the cases, it can be concluded that non-responders had presented with an advanced stage of disease and in case number 21 remission maintenance was very poor. Whether, this has any direct relation with no karyotypic response is too

premature a conclusion, but possibility exists in which a larger study might point in this direction.

The other two cases (numbers 27 and 28) both showed few metaphases, but were not well spread and hence karyotyping was not possible. Both the patient had Hodgkin's lymphoma (HL) and had come in early stages. Probably, this abnormal karyotyping response has no clinical significance.

Chronic myeloid leukemia (CML)

In the present study, the largest group is that of CML. Out of total 40 cases, 12 belong to this group (30.0%). CML as has been already stressed, is the most widely studied neoplasm as far as the cytogenetic aspect is concerned. Philadelphia chromosome which is seen in 90% cases of CML is the first consistent chromosomal aberration detected in any malignancy.

Of the 12 patients, 5 were males and 7 females. The age incidence is between 21 and 60 years, with tendency to develop at a later age. Five of these patients - numbers 1,2,3,5 and 11 were known patients and were studied during recurrence while the other 7 (numbers 4,6,7,8,9,10,12) were studied during their first diagnosis.

In the present series, the numerical aberrations were quite infrequent and the cell lines showed near diploid states. All the cases had chromosome number in the rage of 45 to 47 with over (1 to 12 except 4) one case showing 43 chromosomes - Case No.4.

Amongst the individual chromosome, the gain of chromosome 8 was seen in case number 2, while the gain of chromosome 21 is seen in case number 7 and case number 9. As reported by Mitelman and Levan (1981), gain of chromosome 8 is very well known and was observed in 45.7% of all abnormal cases. Involvement of chromosome number 21 though less frequent, is also reported.

Loss of chromosome is less frequent and was reported in case number 8 as loss of chromosome 6 and in case number 1, the follow up examination showed loss of 15 in one cell line and loss of 18 in the other cell line. The loss of chromosome is not very common and usually is a random finding.

Structual rearrangements are more important in CML. The most well known is the Philadelphia chromosome (Ph^1).

In the present series of 12 patients of CML, 10 showed Ph^1 chromosome which mainly means deletion of one long arm of 22nd chromosome i.e. 22q- condition. The cases are numbers 1,2,3,4,5,6,7,9,10,12 and cases numbers 8 and 11 in the series in acute phase of CML (blastic crisis) did not show Ph^1 chromosome. Thus, 83.33% of the cases in the present series had Ph^1 + cell line. The review by Whang-Peng et al (1968) revealed Ph^1 + cells in 85% of cases.

In the present series due to unsatisfactory banding results translocation from 22 chromosome is not well localised. In case number 5, there appears to be a classical Ph^1 translocation of t(9q+, 22q-) (Fig.44). In case number 1 showed an unusual translocation of t(2q+, 22q-). Involvement of 2nd chromosome in Ph^1 translocation has been previously reported by Hayata et al (1975) and van den Berghe.

As discussed, 95% of total Ph^1 positive CML cases show classical t(9; 22) translocation while only 5% show simple (unusual) or complex Ph^1 translocation.

In case number 1, in the initial stages cytogenetic study revealed 46,XX t(2,22). The same patient was again analysed one year later. This time a new clones in the form of 45,XX, -15,22q/45,XX -18,22q-, del (8) (P) appeared (Fig. 45). The same patient few months later presented with blastic transformation in CML. When last seen she was in a poor condition.

The second interesting case is number 8, who had presented with blastic crisis and died in a week. His karyotype did not reveal Ph^1 chromosome. The only abnormality detected in this case was 45, XY, -6. It also did not show some of the non-random changes reported in the acute phase affecting chromosomes 8,17 and 22 (Mitelman and Levan, 1981).

The important aspect of cytogenetic study of CML is its prognostic value. Presence of Ph^1 cell line is associated with much better prognostic (Whang-Peng, 1968).

In the present study, out of 12 Ph^1+ patients, 10 are alive for longer time. The patient who died, survived for 18 months after the first diagnosis. Other 10 patients are alive for as long as more than 7½ years and 6 years. This finding confirms the long proved theory of better prognosis with Ph^1+ CML. All these patients also had better treatment response and earlier remission induction and a longer remission maintenance. Change in prognosis with different type of Ph^1 translocation could not be studied due to the limitations in methodology. In literature no appreciable difference in survival is noted between complex, unusual or typical Ph^1 translocation.

Case No.8 which was Ph^1 negative had very bad prognosis. This was mainly because of his presentation straight-way in acute phase.

Other important observation was in case number 1. In this case, a change in karyotypic picture was noted two months prior to her reporting in blastic crisis. This finding is in accordance with the observation that karyotypic change might precede the clinical signs of blastic crisis by two to four months (Hayater et al, 1975). It also supports a view that change in the karyotype is a grave prognostic sign (Whang-Peng, 1968).

Chronic Lymphocytic Leukemia (CLL)

The next group is chronic lymphocytic leukemia (CLL). In the present series, 3 patients of CLL were studied cytogenetically. The clinical details of these patients are summarized in Table-17 and Table-18.

As shown in the table, 1 patient had completely normal karyotype (No.15) while the other patient (No.13) had two clones, one normal 46,XY, while other showed 47,XY +20 pattern (Fig.46). The third patient (NO.14) had 45,XX, -15 pattern.

Such type of aneuploidy is frequently reported (Spiers and Baikie, 1968; Crossen, 1975; Nowell et al, 1976; Fleishmann and Prigogina, 1977). The commonest chromosomes to be involved are 12, 14 and 17 (Mitelman and Levan, 1981) though other chromosomes are also involved in random fashion.

In the present series of CLL, no structural aberrations were detected. The commonest structural change reported is a 14q+ abnormality (Mitelman, 1981).

In the present study, all the three patients had reported in a very late stage (III or IV). Case No.15 who had normal karyotype 46,XY had come with complication of haemolytic anaemia and succumbed in 5 days. Both the other cases also had average treatment response. Case No.14 died in a year. From this data, no correlation between the cytogenetic findings and the prognosis or the progression of disease can be made.

As reported in the literature, no correlation with the prognosis and cytogenetic findings is so far proposed mainly because of paucity of information on this aspect and this disease.

Acute Myeloid Leukemia (AML)

In the present series, there were 4 patients with AML (Case numbers 16, 17, 18 and 19). The clinical details are summarized in Table-17 and Table-18.

All the three patients who died reported to the hospital with advanced disease and some associated complications like intracranial haemorrhage and septicemia. Case No.16 and Case No.18 had marked thrombocytopenia, while case number 17 had signs of septicemia on admission. All the 3 had

a very bad prognosis on admission. The fourth patient (No.19) had normal peripheral blood smear but high myeloblast count on bone marrow examination. He had no associated complication.

Among the numerical aberrations only case number 19 revealed a clone with 49 chromosomes where there are two additional ? G group chromosomes and one ? D group chromosome (Fig. 47). All other three cases had normal number of chromosomes.

Case No.16 revealed totally normal pattern in all cells i.e. 46,XX while case number 17 showed mixed picture in which 46,XY, -7, +mar, clone was present with some normal cells with 46,XY pattern. Case No. 19 had normal number of chromosomes (46) but showed structural aberrations.

In the present series, out of 4 cases only 1 had completely normal karyotype, i.e. 25%. In a series of 130 patients reported by Garson (1980) 49% patients had normal karyotype. Loss of chromosome 7, reported in case number 17, is one of the commonest aberration in AML. Mitelman and Levan (1981) reported affection of chromosome number 7 in 22.9% of total 496 cases, while 64.9% of these cases had loss of chromosome 7. Case No.19 showed complex numerical aberration with involvement of chromosome 22, 21 and 13 as addition and chromosome 20 as loss. Involvement of chromosome 21 is reported by Mitelman and Levan (1981) in 23.4% of 496 cases of AML.

Amongst the structural aberrations, case numbers 18 and 19 revealed interesting finding of 22q- change, suggesting the presence of Philadelphia chromosome in these acute leukemia cases. The fate of the translocated piece could not be ascertained due to the poor banding results. Case No.19 also showed some other complex structural rearrangements.

In the present study, of the 3 patients who did not respond to the treatment, had poor prognosis and subsequently died, 1 had normal karyotype (Case No.16). One had mixed population of cells (Case No.18)

and one had presence of Ph^1+ cell line (Case No.17). While the 4th patient with multiple complex aberrations (Case No.19). Though this series is too small for any generalization, apparently the cytogenetic aberrations have not affected the disease process itself or have not influenced the treatment response or prolonged the survival. One of the other factor for bad prognosis may be very late admission of these patients with terminal complications.

Many investigators agree with the above stated view that karyotypic abnormality does not influence the change of leukemic remission or patient survival (Fitzgerald et al, 1976; Fitzgerald and Hamer, 1973 and Guns, 1973). While the opposite view that presence of normal karyotype is associated with longer survival is presented by other authorities (Sukurai and Sandberg, 1973 and 1976; Golomb et al, 1978).

Presence of Ph^1 chromosome in AML also did not change the prognosis in any way. Bloomfield et al (1977) and Golomb et al (1978) reported similar observation.

Acute Lymphocytic Leukemia (ALL)

In ALL totally 3 patients (Case numbers 20,21 and 22) are studied cytogenetically. The clinical details are given in Table-17 and Table-18.

All the 3 patients had marked thrombocytopenia. Case No.20 died due to intracranial haemorrhage.

In case number 21, there was failure to get metaphases for karyotyping.

Case No.20 has completely normal karyotype 46,XY and No.22 has majority of 46,XY clones with one cell line showing 45,XY, -1 +mar pattern.

This basically normal karyotype is reported in upto 50% of total cases of ALL studied so far (Oshimura et al, 1977a; Cimino et al, 1979 and Sandberg, 1980).

In the present study, no structural rearrangements were reported.

Two patients (No.20 and No.22) had very bad prognosis on admission and died in a month. Both the cases had reported late with marked thrombocytopenia while both had majority of cells with normal karyotype, case number 20 had all cells with normal chromosomal pattern.

The survival and initial remission duration were reportedly better in patients with initial normal karyotype (Hart et al,1971 and Cimino, 1979). Contrary to this view, Whang-Peng et al (1976) reported no significant prognostic value to the appearance of aneuploid cells in the bone marrow. Though they acknowledged the fact that persistence of abnormal lines and the development of total aneuploidy signals poor prognosis.

In this study both the cases had normal karyotype with bad prognosis. This probably supports the later view or it may be due to very late presentation. Any conclusion will be too premature.

Hodgkins's Lymphoma (HL)

Of the 40 cases studied, 10 i.e. 25% belonged to Hodgkin's lymphoma group. Summary of the cases is presented in Table-17 and Table-18.

In the present series, amongst the numerical aberrations in HL. Case No.26 and No.28 have normal cell lines i.e. 46,XY; while the other three cases 23,25 and 27 showed cells line with 45, chromosomes. Case No.23 revealed loss of chromosome 18. Case No.25 showed absence of chromosome 1 and case number 27 showed loss of chromosome 2. Random involvement of almost all the chromosomes have been reported, but no consistent numerical change affecting single chromosome is reported.

One of the most significant observation in HL patients was the presence of mosaic population with a clone with hyper diploid state. Of these patients, 25,26,27 and 28 showed cell population with chromosome in the range of triploidy or tetraploidy. Thus, in the present series, 80% cases revealed high model chromosome numbers. Similar findings are reported by

Sandberg and Hossfeld (1979); in their series of 35, out of 48 cases (79.2%) showed high model number. This type of findings suggests the presence of the different modes suggesting clonal evolution of the disease. Even attempts have been made to correlate these findings with the presence of Reed-Sternberg cells.

Amongst structural changes reported in the present series, case numbers 23 and 27 revealed 14q + (?) abnormality. The origin of the added piece in both the cases could not be traced. These findidngs are in conformity with the previously well known reports of the presence of this aberration in all B cell neoplasia including Hodgkin's disease. This finding has been frequently reported by many authors (Reeves, 1973; Lawler, Reeves and Namlin, 1975; Fakuhara, Shirakawa and Uchino, 1976; Fukuhara and Rowley, 1978 and Mitelman and Levan, 1981).

In the present series, no correlation was found between the clinical staging of the disease and histological diagnosis with cytogenetic findings. Also no specific relation was noted with prognosis, treatment response, remission induction and maintenance and survival.

Also in the literature reviewed, no correlation was found between the chromosomal changes and disease progression. Only observation made is the presence of more number of karyotypically normal cell line in lymphocytic predominant and mixed cellularity type of HL than lymphocytic depleted type. This finding is also not confirmed in the present series.

Non-Hodgkin's Lymphoma (NHL)

In this group of NHL, total 6 patients were studied cytogenetically. Their clinical history and other details are summarised in Table-17 and Table-18.

In the present study of 6 cases of NHL majority (5 cases) were males. They belonged to a wide range of group between 10 and 60 years. Case numbers 33, 37 and 38 had histological diagnosis of lymphocytic, poorly differentiated type; while case number 34 had diffuse histiocytic type. All

the four had bad prognosis on the basis of histological type. All these patients died within one year. The other two cases (No.36 and 37) had histological pattern with favourable prognosis.

The case numbers 35,36,37 and 39, all had completely normal karyotype. 46,XY or 46,XY while case numbers 33 and 34 had no numerical aberrations but showed structural rearrangements. Case number 34 also showed added normal cell line 46,XY. Case number 33 is the only case which revealed lymphomatous infiltration of the bone marrow and it can be postulated that this might be responsible for the detection of abnormal clone in these patients.

Amongst the structural rearrangements seen in the present series, case number 33 showed classical 14q+ abnormality (Fig. 48). The probable origin of this added segment is 21st chromosome. In case number 34, an additional clone revealed deletion of long arms of chromosome 3 (Fig. 49)

Presence of 14q+ marker has been reported very frequently in all B cell malignancies and hence also in NHL (Fukuhara and Rowley, 1978; Fukuhara et al, 1978 and Mitelman and Levan, 1981). McCaw et al (1971) even put forward a notion that the structural rearrangements involving chromosome 14 may play a role in the development of lymphoid malignancies. Involvement of chromosome number 3 as seen in case number 34 is frequently seen in NHL. This has been reported by Hossfeld and Schmidt (1978).

For the prognostic significance possibly no conclusion could be drawn as out of 4 patients who died, 2 had normal karyotype, while 2 had abnormal. No correlation with histological type and cytogenetic abnormality is seen. Similar reports are published by numerous authors (Sandberg and Hossfeld, 1974 and Atkin, 1980).

Multiple Myeloma (MM)

In the present series, there were 2 patients of multiple myeloma. Both the cases (numbers 39 and 40) were known patients of MM and were surviving for more than three years. Their clinical history is summarised in Table-17 and Table-18.

In the present study, both the patients showed complex aneuploidy with multiple clones. Case number 39 also showed a clone with normal 46,XY configuration. In the literature, no consistent abnormality is noted and all the chromosomes have been reportedly involved in random fashion.

Amongst the structural changes, besides complex unidentifiable markers, case number 40 showed a large marker chromosome, even larger than group A chromosome (Fig.56). This chromosome is the MG chromosome (Houston et al, 1967). Similar large MG marker was reported by Siebner et al (1969) in 35% of cases in a series of 54 cases. This chromosomal marker is partly formed by chromosome number 1 (Phillip and Drivsholm, 1975). Similar participation of chromosome one is also found during the present study.

Prognostically, no cytogenetic finding has showed any significant influence on the survival. The only consistent observation is that all the numerical and structural abnormalities appear in the later stages of the disease.

In the present study, both the patients had multiple abnormal clones and both were in their late terminal stage. This is consistent with the above observation.

At the end, it can be emphatically stated that in each and every case of haematological malignancy, the study of cytogenetic profile must form an integral part at all the stages - be it the first diagnosis or during the treatment, blast crisis, remission or reactivity. Only such multicentric, prospective well controlled and critically evaluated longitudinal studies carried out in a large number of patients will provide some clue or clues to unravel many more mysteries. Certainly this will play a leading role either in radically changing the understanding of the pathophysiology, progression, prognosis or therapy of these conditions.