CYTOGENETIC STUDIES IN PRIMARY AMENORRHOEA

by

G. VIJAYA LAKSHMY,* M.B.B.S.,

SHAMER SINGH,** B.Sc., M.B.B.S., M.S.

and

D. SHARMA,*** D.G.O., M.S.

The chief function of the two sex chromosomes has been ascribed to be the supervision of primitive germ cells (Jones *et al*, 1963). A full complement of normal chromosomes is necessary for the proper segregation and perhaps migration of germ cells. However, anomalies of the genital organs or gonadal dysgenesis may exist without any chromosomal aberrations. Primary amenorrhoea, a symptom of large number of diverse clinical entities was cytogenetically studied by Jacobs *et al* (1961); Philip *et al* (1965); Jagiello *et al* (1966); Chaudhury *et al* (1966); Bhose *et al* (1967) and Canales *et al* (1971). While these studies contain considerable information relevant to the problem from a cytogenetic view point, it has become evident that there are extreme variations in these patients and as such the understanding of the role of chromosomes in the pathogenesis of primary amenorrhoea has become difficult. Early karyotyping in many of these cases can circumvent both the tedium and expense of repeated futile courses of both hormone studies and therapy.

The present communication is intended to add the results of cytogenetic studies in 10 Indian subjects to the pool of information already available.

*Lecturer, Department of Anatomy.

**Professor & Head, Department of Anatomy.

***Reader, Dept. of Obst. & Gynec.

Institute of Medical Sciences, Banaras Hindu University, Varanasi-5.

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Material and Methods

Out of the 45 cases referred for cytogenetic analysis to our laboratory during 1969—1971, 10 cases had primary amenorrhoea. Their age ranged between 18 and 27 years, the average age of menarche in India being 13-15 years (Purandare 1945; Logambal and Bhaskara Rao, 1969).

Clinical examination: The patients were subjected to thorough clinical examination for cardiovascular and other somatic malformations. Pelvic examination was done in detail (per vaginal and per rectal routes). Laparotomy was carried out in two of these 10 cases. Pelvic pneumography was done in one patient.

Cytogenetic studies: Sex chromatin was studied by buccal smears. Counts less than 2 per cent were considered negative.

Chromosomal analysis: Short term leucocyte cultures were done by the method of Arakaki and Sparkes (1963) in all these cases. To obtain good spreads and flattening, slides were passed over a flame before air drying. Dried slides were stained by carbol fuchsin. For counting and karyotyping the intact cells were chosen with the following criteria.

1. Cells exhibiting the highest degree of chromosome morphologic acuity.

2. Cells representing mitotic metaphase.

3. Cells having the least amount of overlapping of chromosomes.

Sixteen cells were counted in each case and wherever there was doubt, 25-50 cells were counted. Photomicrographs were taken for all the cases and the chromosomal complement was reassessed and morphology was studied after karyotyping which was done according to Denver Convention (1960).

Observations

Cytogenetic analysis done in ten cases of primary amenorrhoea showed all of them to be positive for sex chromatin and the cells showing Barr bodies ranged between 15-45 per cent. Each subject demonstrated a modal chromosomal number of 46 with 16 in the 6-12 + X group and was found to have a normal female karyotype. Clinical and cytogenetic data are shown in Tables I and II respectively.

TABLE I

Showing Clinical Features in the Cases of Primary Amenorrhoea

Case No.	Age in years	Secondary sex characters	External genitalia	Internal genitalia
1	20	Well developed	Normal	Uterus absent. Vagina absent
2	19	Well developed	„	Ovaries normal size. Blind vaginal pit present. Laparotomy showed absence of uterus. Ovaries normal size. Pelvic kidney on the right side.
3	25	Well developed	„	Vagina 3 cm. depth. Uterus absent. Pelvic pneumography confirmed absence of uterus. Ovaries normal size.
4	20	Well developed	„	Transverse band present in the place of uterus. Vagina absent.
5	21	Underdeveloped	„	Uterus and vagina present. Ovaries cystic on both sides.
6	20	Well developed	„	Vagina 1 cm depth. Uterus absent. Ovaries normal on both sides.
7	20	Well developed	„	Uterus grossly hypoplastic. Vagina 1½ cm. Ovaries normal on both sides.
8	18	Axillary hair absent Pubic hair scanty (Fig. 1)	„	Uterus and vagina absent (Fig. 2) Ovaries present but smaller in size.
9	19	Underdeveloped	„	Uterus present. Vagina present. Ovaries present on both sides but small.
10	20	Underdeveloped	„	Uterus and vagina present. Small sized ovaries on both sides.

TABLE II
Summary of Cytogenetic Data

Case No.	Phenotype	Sex Chromatin + %	No cells counted	Chromosomes 45	46	47	Karyotype
1	F.	36	16	0	16	0	46, XX
2	F.	40	16	0	16	0	46, XX
3	F.	44	50	0	50	0	46, XX
4	F.	45	16	0	16	0	46, XX
5	F.	38	16	0	16	0	46, XX
6	F.	34	16	0	16	0	46, XX
7	F.	42	16	0	16	0	46, XX
8	F.	42	16	0	16	0	46, XX (Fig. 3)
9	F.	38	16	0	16	0	46, XX
10	F.	15	25	0	25	0	46, XX

Discussion

Patients presenting with the leading symptom of primary amenorrhoea may be clinically divided into two groups:

1. Gonadal dysgenesis with normal female differentiation of Mullerian ducts.
2. Developmental defects of the organs derived from the Mullerian ducts with fairly normal gonads.

Out of the ten cases in the present series, 3 cases (case No. 5, 9 and 10) belonged to the former group. Case No. 5 had abnormal cystic ovaries while case No. 9 and 10 had hypoplastic ovaries.

Various types of chromosomal abnormalities from numerical aberrations to structural abnormalities have been reported in cases of primary amenorrhoea with gonadal dysgenesis (Jacobs *et al*, 1961; Philip *et al*, 1965 and Jagiello *et al*, 1966). However, Chaudhury *et al* (1966) and Bhose *et al* (1967) have reported normal chromosomal patterns in primary amenorrhoeics with gonadal dysgenesis. Ovarian dysgenesis is thought to be due to defects of sex chromosomal complement (Jones *et al*, 1963). They claimed that the chief function of the active sex chromosome may be the supervision of the activity of primitive germ cells. Cases 9 and 10 of the present series had gonadal dysgenesis in the form of hypoplastic ovaries but had normal chromosomal complements. Gonadal dysgenesis in these cases could have been due to point mutation which cannot be detected by the methods of chromosome analysis available at present. It might also be due to concealed mosaicism (Bhose *et al*, 1967) or viral infection (Jones *et al*, 1963) or other environmental factors. Although the chromosomal analysis of 25 cells in case No. 10 did not reveal any detectable abnormality, the low sex chromatin count (15 per cent) probably is suggestive of concealed mosaicism.

The possibility that the sex chromosomal abnormalities play a role in the pathogenesis of the syndrome of primary amenorrhoea associated with cystic ovaries became evident by the discovery of chromosomal anomalies in these types of cases by DeGrouchy *et al* (1961). But case No. 5 in this series, with cystic ovaries, had normal female karyotype and is similar to the cases reported by Teter (1967) and Canales *et al* (1971). Further improvements in the techniques to find minute chromosomal defects might throw more light in these types of cases.

Rest of the seven cases belonged to the second group. Their phenotypic sex was female. All had well developed secondary sex characters except case No. 8 which showed absence of axillary hair and scanty pubic hair, but with well developed breasts. All these cases were positive for sex chromatin and had normal female karyotypes with a modal number of 46 chromosomes with 16 in the 6 12-X group (Fig. 1). The studies of earlier workers (Azoury *et al*, 1966; Capraro *et al*, 1969; and Grover *et al*, 1970) have not revealed any abnormalities in the chromosomal complements of cases with developmental defects of Mullerian ducts and our findings fall in line with these reports. Though the available literature does not show any interrelationship with the differentiation and development of Mullerian ducts and chromosomes all the same it is advisable to do cytogenetic analysis in all suspected cases of vaginal agenesis and confirm chromosomal sex before doing any reconstruction surgery in such cases. The futility of subjecting the patient to laparotomy for ruling out testicular feminisation syndrome can be avoided by cytogenetic study.

Summary

1. Ten cases of primary amenorrhoea were studied cytogenetically (Sex chromatin and chromosome analysis).
2. Three cases were of gonadal dysgenesis and 7 were with developmental defects of Mullerian ducts.
3. All the ten cases had normal female karyotypes without any detectable structural abnormalities.
4. Chromosomal sex must be confirmed before proceeding with reconstructive operations of vagina in cases of Mullerian duct developmental defects.
5. Early karyotyping can circumvent both the tedium and expense of repeated futile courses of hormone therapy and hormone studies in many cases.

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See Fig. on Art Paper II