STUDY OF SEX CHROMATIN IN CONGENITAL MALFORMATIONS OF THE GENITAL TRACT

by

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Introduction

Genital tract malformations are quite commonly seen in gynaecological practice. Most of the cases are diagnosed in the second decade of life presenting for the treatment of primary amenorrhoea or primary infertility. Some of the cases of gross abnormality as in true hermaphroditism are diagnosed relatively early. Cases of uterine malformation present for the treatment of repeated abortion, stillbirth or with recurrent abnormal foetal presentations. Prognosis in some cases is doomed; but, on the other hand, in some cases surgical correction is the choice of treatment. Nuclear sexing in such cases has a prognostic value. A phenotypic female may have male type of chromosomal pattern. Thus nuclear sexing is a very important investigation in cases of genital tract malformations. It is an important tool to differentiate cases that can be benifited by surgical treatment from those who cannot. Further, it gives an idea about the type of gonads present because the nuclear sex goes with the gonadal sex. By doing nuclear sexing many unwanted laparotomies can be avoided. Of course, now-a-days, the laparoscope has made it easier to see the type of gonads present and to take a punch biopsy for confirmation. Ideally speaking, every case of genital tract malformation should have a complete chromosomal study. Karyotyping is quite elaborate and is possible only in the institutions and big hospitals. Study of sex chromatin is very easy and can be done anywhere. It gives an idea about the chromosomal pattern on karyotyping. In the present work, sex chromatin was studied in 40 cases of primary amenorrhoea which include 28 cases of genital tract malformations.

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Material and Method

Chromatin study was carried out in 50 normal healthy females and 30 males for control purpose. Forty cases of primary amenorrhoea were studied. Apart from chromatin study I.V.P., gonadal biopsy, x-ray chest and of pituitary fossa were done when indicated.

Buccal mucosa smear was collected for chromatin study. The subjects were requested not to chew betal or nuts for few days before collection of tissue. Preliminary mouth wash with warm saline was advised to avoid bacterial contamination. Tissue was collected by scraping the inner side of cheek and was smeared on a clean slide which was plunged immediately in ether and alcohol mixture—the fixative. Staining method—The cresyl echt violet method of Moore (1962) was employed. It is the best and most reliable method.

Appearance of Sex Chromatin: Sex chromatin was seen as a deeply stained dot in the nucleus lying against the nuclear membrane (vide figure). Only

those cells which fulfilled the following criteria were taken into account.

(a) Cells with large circular and lightly stained nuclei.

(b) No crenation of the nuclear membrane and no undue folding of the cell membrane.

(c) No overlapping of cells.

(d) Free from bacteria.

(e) Plano-convex deeply stained bodies were considered. In each slide at least 200 cells were counted and the number of sex chromatin was expressed in percentage.

Observation

In the control group, majority of the women had 31-40% sex chromatin and in men it was 0-2% (vide Tables I and II). The age group was 15-20 years in 75% of subjects studied.

Aetiologically, the cause of primary amenorrhoea was due to chromosomal anomalies, congenital anomalies of the genital tract, tuberculous endometritis and delayed menarche (Vide Table III).

Out of 40 cases of primary amnorrhoea, 34 were chromatin positive and 15 were chromatin negative. Congenital malform-

TABLE I

Sex Chromatin Percentage in Normal Females (Positive Control)

	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80
Number of cases	—	—	16	24	6	2	2	—
Percentage	—	—	32	48	12	4	4	—

TABLE II

Percentage of Sex Chromatin in 30 Normal Males (Negative Control)

	Percentage of sex chromatin				
	0-2	3-5	6-8	9-11	12 or more
Number of cases	26	4	—	—	—
Percentage	86.66	13.33			

TABLE III

Aetiological Classification of Primary Amenor hoea

Aetiology	Number of cases	Percentage
(a) Chromosomal anomalies	6	15
(b) Congenital anomalies of the genital tract	25	62.5
	3 chromatin negative cases are included in group 'a'	
(c) Tuberculous Endometritis	1	2.5
(d) Delayed menarche	3	7.5
(e) Positive withdrawal therapy	4	10
(f) Follow-up could not be done	1	2.5

ation of the genital tract was found in 28 cases, out of which 3 cases were chromatin negative. All of these 3 chromatin negative cases had vaginal atresia (Vide Table IV).

In the present series, out of 40 cases of primary amenorrhoea in 28 cases the amenorrhoea was due to congenital anomalies of the geintal tract like vaginal atresia, absence or gross hypoplasia of uterus, cervical phimosis and imperforate hymen etc. (Table IV). Out of these 28 cases, 25 were chromatin positive and 3 were chromatin negative. All these 3 chromatin negative cases had gonadal dysgenesis which was known by gonadal biopsy. Kunwar *et al* (1966) reported congenital malformation responsible for primary amenorrhoea in 47.85% of cases.

TABLE IV

Distribution of Congenital Malformations of Genital Tract in 28 Cases of Primary Amenorrhoea

Congenital malformation	No. of cases	Nuclear sex
Vaginal atresia	19	Chromatin +ve in 16 and —ve in 3 cases
Gross hypoplasia of the uterus	7	All +ve
Imperforate hymen	1	+ve
Cervical phimosis	1	+ve

Discussion

Moore and Barr in 1952 demonstrated the sex chromatin in human cells. According to them 40% cells should show chromatin body in chromatin positive individuals. In the present study, majority of normal women had 31-40% chromatin +ve cells. Keith, Moore and Barr (1955) for the first time reported their observation on sex chromatin in buccal mucosa and discovered the superiority of buccal mucosa over many other tissues. It has become the specimen of choice for chromatin study due to easy collection, good number of cells show chromatin body and not much experience in cytology is needed for interpretation.

Sex chromatin tests are the most valuable adjunct to direct examination of chromosomes. It is well known that number of Barr bodies is always one less than the number of X chromosomes present. Thus by studying the sex chromatin the number of X chromosomes present can be guessed. Though Karyotyping is ideal in each case it is not possible everywhere. Thus a working idea can be obtained by this simple method of chromatin study.

Upadhyay (1954) reported 19 cases of congenital anomalies of genital tract out of which 9 had primary amenorrhoea and in 7 out of the 9, amenorrhoea was due to vaginal atresia.

According to Stevenson (1966) the majority of microscopic developmental disorders in the body are not known to be associated with chromosomal abnormality. Ramez *et al* (1966) studied 12 cases of congenital absence of vagina and all were chromatin positive. When these developmental disorders affect the genitalia they mimic or indeed be indentical with some of the associated karyotypic anomalies. However, majority appear to be part of general instability of development of structure derived from the urogenital ridge, so that there are associated

urinary tract malformations as well. In the present series, no case had associated urinary tract malformation.

Thus, chromatin study is an easy and important investigation in malformations of genital tract and can be used as a screening tool for further management. It gives an idea about the chromosomal pattern and the gonadal sex.

Summary

(1) Sex chromatin study was carried out in 40 cases of primary amenorrhoea out of which 28 had genital tract malformation.

(2) 25 cases of genital tract malformation were chromatin positive and 3 were chromatin negative.

(3) The importance of this simple test has been stressed because it is an adjunct to karyotyping.

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See Fig. on Art Paper V