

# Cytogenetic Study In Patients With Menstruation Disorders

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## Abstract

Primary amenorrhea, secondary amenorrhea, oligomenorrhea and hypomenorrhea are usually referred for cytogenetic evaluation. The study was conducted over a period of 24 months. The patients diagnosed by clinical departments as menstruation disorder cases were included in the study. The chromosomes were obtained using conventional short term culture method. There was a striking finding of 46XY patients in 6 cases. Only 2 patients were seen with Turner syndrome. The remaining 7 patients had mosaicism. Only one patient had isochromosome. Aims: The study was undertaken to evaluate the chromosomal causes and their frequency in relation to menstruation disorders. The authors wanted to compare the data of this study with previous studies. Settings and Design: Hundred cases referred for the complaints of menstruation disorders were included in the study which was conducted over a period of 24 months. Methods and Material: Chromosomes were obtained through conventional short term culture method. Karyotype was prepared and analyzed after arranging the chromosomes according to Denver classification. Statistical analysis used: Nil. Results: Out of 100 patients 16 showed abnormal chromosome complement. Other patients had normal complement. There were six cases with 46XY complement. Only two patients had Turner syndrome. Remaining seven patients had mosaicism and one patient had isochromosome. Conclusions: Male chromosomal complement has been discovered in normal looking phenotypic females in present study. Therefore it is recommended that Cytogenetic study be carried out in evaluating cases of amenorrhea.

## INTRODUCTION

Few problems in gynecologic endocrinology are as taxing and challenging to clinician as Amenorrhea.

Primary amenorrhea, secondary amenorrhea, oligomenorrhea and hypomenorrhea are usually referred for cytogenetic evaluation. The incidence of primary amenorrhea is 10% and that of Secondary amenorrhea is 30-32 %.<sup>[1]</sup>

Till Year 2001 chromosomal abnormalities were recognized in 50% cases of spontaneous abortions and 25% of these had major abnormalities. The most common abnormalities in abortuses were 45, X which is one of the major cause of menstruation disorders presenting as primary amenorrhea. The incidence of this abnormality is 1 in 2500 live female births.<sup>[2]</sup>

Mullerian duct abnormalities (incidence 1 in 4000 live female births) and Testicular feminization syndrome (incidence 1 in 2000 births) are the second and third most common causes of primary amenorrhea.<sup>[3]</sup>

## ETIOLOGICAL FACTORS

Various etiological factors are responsible for menstruation disorders including physiological factors (pregnancy, lactation, menopause and before menarche), and pathological factors affecting hypothalamus, pituitary, thyroid, ovaries, adrenals, uterus and nutrition.

## DEVELOPMENTAL ASPECTS:

Sexual differentiation in embryo is achieved at 12 to 15 weeks of gestation. The developing gonad is bipotential till 6 weeks. The critical role of Y chromosome and of male hormones is well documented. Genetic sex depends upon the type of sperm fertilizing the ovum; the type of gonad that develops is determined by the sex chromosome complement (XX or XY). The development of indifferent gonad into a testis depends upon the presence of Y chromosome. In its absence female gonads develop irrespective of number of X chromosomes. This is due to presence of testis determining factor located in SRY region of short arm of Y chromosome.

Translocation of this SRY region of Y chromosome in meiosis I of spermatogenesis leads to XX males and XY females. Also in case of Turner syndrome majority of XO zygotes occur due to post zygotic error with loss of paternal sex chromosome (Around 70 to 80% patients with 45X

complement).

## REVIEW OF LITERATURE

The credit of discovery of cases presenting with short stature, post pubertal age with ovarian dysgenesis having primary amenorrhea, absent ovaries and absent pubic and axillary hairs goes to H. H. Turner who described these cases in 1938.

Historical background of secondary amenorrhea is difficult to trace back but as mentioned by Speroff et al <sup>[5]</sup> the first case of secondary amenorrhea was seen 1000 years ago where 7<sup>th</sup> daughter of king of Portugal turned to intense prayer in her protest against arranged marriage which led to anorexia and secondary amenorrhea with hirsutism.

Since then this topic has been studied by many authors from time to time.

## MATERIALS AND METHODS

Among different cases referred to the OPD of this Institute 100 cases referred for the complaints of menstruation disorders were included in the study which was conducted over a period of 24 months. The study was carried out after permission from institutional ethics committee.

The history and the findings of the clinical examination were noted down in the proforma prepared by the department.

Using all aseptic precautions, 2 ml venous blood was taken in a sterilized preheparinized vacutainer. This was placed in the refrigerator at 4-8°C. Culture was set in laminar air flow chamber fitted with ultraviolet light under all aseptic precautions. Around 8 drops of blood was added to the culture vial containing 5ml RPMI 1640. To this 0.2 ml of phytohaemagglutinin was then added along with streptomycin and penicillin (1 ml each). The Culture vial was sealed tightly, labeled and incubated at 37°C for 72 hours. After 69 hours of incubation 0.2 ml of colcemid was added to the culture and incubated for 90 minutes. The culture contents were transferred to centrifuge tubes and centrifuged for 10 minutes at 1000 rpm. The supernatant was then discarded. The cell pellet was resuspended with 5 ml of prewarmed hypotonic solution and allowed to stand for 10 minutes at 37°C. This was centrifuged for 5 minutes at 1000 rpm. Supernatant was then discarded & cell pellet resuspended in 5 ml freshly prepared fixative fluid (methanol: glacial acetic acid: - 3:1). The cells were resuspended. This cell suspension was refrigerated at 4-8°C and after an hour, this was centrifuged. Several such changes

were given till a clear cell suspension was obtained. This suspension was then dropped on to a clean, sterile chilled slide from a height of 1 foot, these slides were then dried, labeled and kept in incubator for 3 days and stained with Giemsa stain. The stained slides were screened for metaphases under low power first and then under oil immersion. Minimum 25 to 30 metaphases were counted and observed.

One good photograph of chromosomes was selected for karyotyping.

## RESULTS

**Figure 1**

Table no. 1: Incidence of different menstruation disorders

Primary amenorrhea	Secondary amenorrhea	Oligomenorrhea	Hypomenorrhea	Total
74	20	4	2	100

**Figure 2**

Table no. 2 : Age wise distribution of patients

Age	Primary amenorrhea	Secondary amenorrhea	Oligomenorrhea	Hypomenorrhea	Total
13-20	57	5	2	2	66
21-25	11	7	1	0	19
26-30	4	7	1	0	12
31-35	2	1	0	0	3
Total	74	20	4	2	100

**Figure 3**

Table no.3: Incidence of abnormal karyotype

Karyotype	Primary amenorrhea	Secondary amenorrhea	Oligomenorrhea	Hypomenorrhea	Total
Normal	61	17	4	2	84
Abnormal	13	3	0	0	16

**Figure 4**

Table no. 4: Incidence of different abnormal karyotypes

Karyotype	Primary amenorrhea	Secondary Amenorrhea	Total
46XY	6	0	6
45X	2	0	2
46XiXq	1	0	1
Mosaics	4	3	7
Total	13	3	16

**Figure 5**

Table no. 5: Clinical findings in patients with 46, XY karyotypes

Features	1	2	3	4	5	6
Age	30	17	18	17	35	18
Cubitus valgus	Ab	Ab	Ab	Ab	Ab	Ab
Stature	N	N	Short	N	N	N
Neck	N	N	Webbed	N	N	N
Sec sex chara <sup>1</sup>	Under	N	Ab	Under	N	Under
Uterus	Ab	Under	Under	Under	Ab	Under
Ovary	Ab	Ab	Under	Testis	Ab	N
Barr body	Neg	Neg	Neg	Neg	Neg	Neg

N= Normal Under= Underdeveloped Ab=Absent Neg= Negative  
= secondary sex characters

**Figure 6**

Table no.6: Clinical findings in patients with 45, X karyotypes

Features	1	2
Age	18	17
Cubitus valgus	Ab	Ab
Stature	Short	Short
Neck	Webbed	N
Sex characters	Under	N
Uterus	Under	Under
Ovary	Ab	?Ab
Barr body	Neg	Neg

N = Normal Under = Underdeveloped Ab =Absent Neg = Negative  
Sex characters = secondary sex characters

**Figure 7**

Table no.7: Clinical findings in patients with mosaicism

Karyotype	Category	Cubitus valgus	Stature	Neck	Sex chara	Uterus	Ovary	Barr body
46XY / 45X	PA	Ab	Short	N	Under	Ab	Ab	Neg
46XY / 45X	SA	Ab	N	N	Under	Under	Under	Neg
46XX / 45X	PA	Present	Short	Webbed	Under	Under	Ab	Posi
46XX / 45X	SA	Ab	Short	N	Under	N	N	Posi
45X / 46XX	PA	Ab	Short	N	Under	Under	Under	Neg
46XX / 45X / 46XIXq	SA	Ab	N	N	N	N	N	Posi
46XX / 45X / 46XX	PA	Ab	N	N	Under	N	N	Posi
46XX / 45X	PA	Ab	Short	N	Under	Under	Ab	Posi

PA = Primary amenorrhea Under = Underdeveloped Ab=Absent Neg = Negative SA = Secondary amenorrhea N = Normal Sex chara= Secondary sex characters

## DISCUSSION

Studies done on patients having menstruation disorders reveal wide range of abnormal chromosomal complements. The findings of present study are compared with the studies done previously.

**Figure 8**

Table no.8: Comparison of age range given by different Authors

Amenorrhea type	Petterson et al <sup>[6]</sup> 1973	Dutta et al <sup>[7]</sup> 1987	Dubey et al <sup>[8]</sup> 2002	Marakchi et al <sup>[9]</sup> 2005	Present study
Primary	NS	16-26	16-35	18-30	13-35
Secondary	25-29	NS	NS	NS	21-30

NS = Not studied.

As it is evident from table no 8 and table no. 1 that maximum number of patients (total 66) between age group of 13-20 have reported to a clinician. Also age of presentation for primary amenorrhea group is early compared to other categories. Patients falling in age group of 21-25 had waited for spontaneous commencement of menses and patients in age group of 25-35 had presented with primary complaint of Infertility rather than amenorrhea. These findings match with findings of other authors.

**Figure 9**

Table no. 9: Comparison of percentage of abnormal karyotypes observed by different authors

	Reindollar et al <sup>[10]</sup> 1981	Mulye et al <sup>[11]</sup> 1983	Opitz et al <sup>[12]</sup> 1983	Midro AT et al <sup>[13]</sup> 1990	Temocin K et al <sup>[14]</sup> 1997	Present study
Total patients	252	120	103	150	77	100
Abnormal karyotypes	40	27	25	61	19	16
Percentage	15.87	22.5	24.27	40.7	24.67	16

From table number 9 and 3 it is evident that the incidence of abnormal karyotype found in this work is similar to studies done by other authors. Also, frequency of abnormal karyotypes is more in primary amenorrhea group than in other categories.

As it is observed from result table number 4 there was a striking finding of 46, XY in six patients of primary amenorrhea group. Rigo et al<sup>[15]</sup> also reported such a high incidence of 46, XY in their study.

Table number 5 shows the clinical features of these XY females. These phenotypic females could be cases of translocation of SRY gene from Y chromosome to X chromosome or there could be Androgen target cell abnormality. Sometimes due to defects in antimullerian hormone, mullerian duct elements might persist with 46 XY complement.<sup>[16]</sup> These cases were referred back to clinician

for appropriate treatment.

It is to be noted that there is absence of Sertoli cells in female patients with XY complement having testicular tissue. There is lack of androgen binding protein and deficient local concentration of the androgen. This leads to failure of maturation of spermatogonia and proliferation of germ cells leading to neoplasia. Hence gonadectomy is advised.<sup>[17]</sup> One such case was discovered in present study that was referred back to clinician for gonadectomy.

Table number 6 shows clinical features of Turner patients seen in present study. The percentage of patients with Turner syndrome is less than the percentage of mosaics found which is shown in Table no.7. It is documented that around one percent Turner fetuses are viable and around 50% of them show mosaicism. Therefore to increase the chances of viability there is a need for mosaicism.<sup>[18]</sup>

As seen from table number 4 there was one patient with isochromosome (46,XiXq). It is well documented that the most common structural abnormality of X chromosome in Turner syndrome is presence of isochromosome.<sup>[19]</sup>

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