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ORIGINAL ARTICLE

46XY Disorder of Sexual Development in Menstrual Dysfunction

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Abstract
Context Any deviation of the normal prenatal development right from gametogenesis may give rise to condition now known as disorders of sex development. XY gonadal dysgenesis is characterized by 46,XY chromosome complement with female phenotype and streak gonads. This study reports incidental finding of eight such cases.
Aim To find out correlation between clinical findings and chromosome complement in cases presenting with menstrual dysfunction.
Settings and Design Prospective, cross-sectional study conducted in a tertiary healthcare center.
Materials and Methods Chromosomes were studied after planting and harvesting through conventional short-term culture method. Attempt was made to correlate the cytogenetic findings with other clinical findings of cases.
Statistical Analysis Nil.
Results Hundred cases of menstrual dysfunction referred from other clinical departments were studied. Abnormal chromosomes were obtained in total ten cases where 46XY disorder of sex development was observed in eight cases and two- cases had Turner syndrome. It was observed that these cases belonged to primary and secondary amenorrhea only. There were six cases where the chromosomal complement was 46,XY and two cases with mosaicism of 46,XY/45,X. Patients with hypomenorrhea and oligomenorrhea had normal chromosomal findings.
Conclusions It is possible to have normal looking females with normal development of secondary sex characters to have abnormal chromosome complement. Cytogenetic testing becomes inevitable in such cases. If possible, molecular diagnostic methods also can be employed for detailed description of derangement.

Keywords Amenorrhea · Cytogenetic · Disorders of sex development · Turner syndrome

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Introduction

Menstrual dysfunction in the form of primary or secondary amenorrhea, oligomenorrhea, and hypomenorrhea poses a tremendous challenge when it comes to diagnosis and management. The etiological correlation between menstrual dysfunction and disorders of sex development (DSDs) is well established. This condition where genotype and phenotype do not match was previously labeled as pseudohermaphroditism. The term "hermaphrodite" derives from Hermaphroditus, the son of Hermes and Aphrodite in Greek mythology, who was fused with a nymph, Salmacis, resulting in one individual possessing physical traits of both sexes.

As a result of discussions at the International Consensus Conference on Intersex, it is proposed that term "disorder(s) of sex development" (DSD) to replace the previously used terms "pseudohermaphroditism," "intersex," and "sex reversal" [1].

There are a number of deviations resulting in disorders of sex differentiation.

The process of sex differentiation is well understood now and is discussed shortly [2].

The process begins with formation of urogenital ridge on posterior abdominal wall of the fetus where future kidneys and gonads develop. Some of the factors like Wilms' tumor suppressor (WT1) and steroidogenic factor 1 (SF1) are required for development of urogenital ridge.

The sexual differentiation is achieved at 12–15 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 on 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 (sex-determining region of Y chromosome) 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. The SRY gene located on Yp11 codes for a protein (high mobility group box) that directs cells called presertoli cells to form testis cords (medullary cords) in the urogenital ridge. Another factor called as SOX9 gene, located on human chromosome 17 is a highly conserved HMG family member and maintains the differentiation of Sertoli cells.

According to studies done on mice, combined effect of Homeodomain transcription factors called Pax2 (paired box genes, written this way as the study was done in mice), Pax8, and Emx2 are required for both formation and maintenance of the male reproductive tract. Another Homeodomain transcription factor called Lim1 is important in case of both male and female reproductive tract development and is required in the developing epithelium of the oviduct and uterus. In addition, Pax2 and Pax8 are thought to be required for mesenchyme-to-epithelium transitions in Wolffian duct formation, and formation of nephron. Retinoic acid signaling and Wnt gene family members are also important for formation and maintenance of Mullerian ducts [3].

In males, Mullerian duct system forms initially, but later regresses because of influence of Mullerian-inhibiting substance which is a transforming growth factor β family member and is secreted by Sertoli cells. As fetal ovaries do not produce this substance, Mullerian ducts persist in females.

The signaling of Mullerian-inhibiting substance is mediated by its type II receptor called Misr2 which is expressed in mesenchyme of Mullerian duct. This Mullerian-inhibiting substance binds with this receptor and causes degeneration of Mullerian duct system. Another factor called hepatic nuclear factor 1 is essential for the formation of Mullerian ducts in human. Persistent Mullerian duct syndrome is a form of autosomal recessive male pseudohermaphroditism where male patients have testes and are virilized but retain ectopic female reproductive tract organs [4].

XY gonadal dysgenesis, or Swyer syndrome, is a type of hypogonadism in a person whose karyotype is 46,XY. The person is externally female with streak gonads and who, left untreated, will not experience puberty. Such gonads are typically surgically removed, and a typical medical treatment would include hormone replacement therapy with female hormones. Mixed gonadal dysgenesis is a condition of unusual and asymmetric gonadal development leading to an unassigned sex differentiation. A number of differences have been reported in the karyotype, most commonly a mosaicism 45,X/46,XY [5].

Mixed gonadal dysgenesis may be interpreted as a specific variation of Turner syndrome. The phenotypical expression depends upon extent of the mosaicism.

Materials and Methods

This prospective, cross-sectional study was carried out between August 2004 and August 2006 after obtaining the approval of the institutional ethics committee. The cases were studied after obtaining a written informed consent.

All cases referred from the Department of Obstetrics and Gynecology, Medicine and Endocrinology of the Institute for Complaints of Amenorrhea and with or without infertility were studied.

The cytogenetic testing is routinely done in this laboratory. The history with special reference to age of menarche in the patient and the history of detailed menstrual record in the case of mother were noted. The findings of the clinical examination were noted down in the proforma prepared by the department.

Two milliliters of blood was collected after peripheral venepuncture in a heparinized syringe using 20 number needle and stored in the refrigerator for planting.

Eight drops of blood were added to a vial containing culture medium having RPMI 1640, fetal calf serum, Heparin, streptomycin, penicillin, and PHA (Phytohemagglutinin). The contents of the vial were mixed gently and were incubated for 69 h at 37 °C.

At the 69th hour, colchicine was added to culture, and vials were again incubated for 90 min after which culture contents were transferred to test tube and treated with hypotonic solution. This was followed by incubation for 10 min. Five milliliters of chilled fixative was added to tube. Several treatments with chilled fixative were given until a clear white cell pellet was obtained. This cell pellet was then suspended in fixative and 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 metaphase under low power first and then under oil immersion. Minimum 25–30 metaphases were counted and observed. One good photograph of chromosomes was selected for arranging the chromosomes according to Denver classification.

Results

One hundred cases were studied in the above mentioned time period. The aim of this article is to lay emphasis on abnormal chromosome complement. The different abnormal complements observed in the study are shown in Table 1. As seen from this table, only one case has secondary amenorrhea, who subsequently was shown to have mosaicism.

Tables 2 and 3 show clinical findings in these cases.

Table 1 Incidence of different abnormal chromosomal complement

Karyotype	Primary amenorrhea	Secondary amenorrhea	Total
46XY	6	0	6
46XY/45X	1	1	2
Total	7	1	8

Discussion

Disorder of sex development (DSD) is a broad category, and it requires thorough understanding of the process of development to pin point the step or the enzyme causing the defect. So far as treatment is concerned, the time of presentation to a clinician is crucial. Some of the cases in the present study have presented in time for appropriate intervention to be done. However, most of the cases in the present study have approached the physician rather too late. In India, the lack of awareness of the issue, hesitancy in disclosing the condition due to social reasons, and financial restrictions are to be blamed for late presentation of such cases.

The Table 1 shows that out of eight, seven patients presented with primary amenorrhea. It is to be remembered that XY mosaicism is often associated with turner syndrome as mentioned previously. In such patients, there is rapid loss of ovarian follicles leading to primary amenorrhea and lack of secondary sex character development. If loss of follicles is after puberty, then the extent of adult phenotypic development and time of onset of secondary amenorrhea will vary accordingly [6]. Hence, it is possible to have secondary amenorrhea and not primary, with abnormal chromosome complement.

Table 2 shows clinical features of these XY females. It was detected on sonography that case numbers 1, 5, and 7 neither had Mullerian duct elements nor did they have Wolffian duct structures. This points to 46,XY gonadal dysgenesis—a heterogeneous disorder that results from deletions or point mutations of SRY gene, duplication of the DSS locus on X chromosome, or mutations in autosomal genes. Some autosomes are also related to DSD. The human 9p deletion syndrome is characterized by variable degrees of 46,XY DSD (female genitalia to male external genitalia with cryptorchidism associated to agonadism, streak gonads or hypoplastic testes, and internal genitalia

Table 2 Clinical findings in cases with 46,XY chromosomal complement

Features	1	2	3	4	5	6
Age	30	17	18	17	35	18
Cubitus valgus	Absent	Absent	Absent	Absent	Absent	Absent
Stature	Normal	Normal	Short	Normal	Normal	Normal
Neck	Normal	Normal	Webbed	Normal	Normal	Normal
Sec. sex. chara.	Under	Normal	Absent	Under	Normal	Under
Uterus	Absent	Under	Under	Under	Absent	Under
Ovary	Absent	Absent	Under	Testis	Absent	Not seen
Barr. body	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Endocrine	ND	↑ FSH LH ↑ Estro. Prog.	↑ FSH LH ↓ Estro. Prog.	ND	ND	ND

Neg. negative, *sec. sex. chara.* secondary sex characters, *ND* not done, *estro.* estrogen, *proge.* progesterone, *under* underdeveloped

Table 3 Clinical findings in patients with mosaicism

Features	7	8
Karyotype	46XY/45X	46XY/45X
Category	PA	SA
Cubitus valgus	Absent	Absent
Stature	Short	N
Neck	N	N
Sex. chara.	Under	Under
Uterus	Absent	Under
Ovary	Absent	Under
Barr. body	Neg.	Neg.
Endocrine	↑ FSH LH ↓ Estro. Prog.	↑ FSH LH

N normal, *under* underdeveloped, *absent* absent, *neg.* negative, *sec. sex. chara.* secondary sex characters, *ND* not done, *estro.* estrogen, *proge.* progesterone

disclosing normal Mullerian or Wolffian ducts) mental retardation and craniofacial abnormalities [7].

Case number 4 had the presence of testicular tissue. This case could be of androgen insensitivity syndrome.

Androgen insensitivity syndromes probably represent the most common identifiable cause of male pseudohermaphroditism. It is a disease connected with the inactivation of androgen receptors due to a mutation that inactivates male sexual differentiation, and causes a spectrum of phenotypic anomalies having, as a common aspect, the loss of reproductive characteristics. The human androgen receptor (AR) is a protein coded by a gene located on the proximal long arm of the X chromosome (Xq11–Xq12) [8].

AIS is divided into three categories that are differentiated by the degree of genital masculinization—complete androgen insensitivity syndrome is indicated when the external genitalia is that of a normal female, mild androgen insensitivity syndrome is indicated when the external genitalia is that of a normal male, and partial androgen insensitivity syndrome is indicated when the external genitalia is partially, but not fully masculinized [9].

It is to be noted that there is the absence of Sertoli cells in female patients with XY complement having testicular tissue (case number 4). 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 advisable [10]. This case was referred back to clinician for gonadectomy.

Sometimes, owing to defects in antimullerian hormone, Mullerian duct elements might persist with 46,XY complement [11] as seen in case numbers 2,3,4, and 6.

It is reported that although specifically male, the human Y chromosome may be observed in female karyotypes, mostly in women with Turner syndrome stigmata. In women with isolated gonadal dysgenesis but otherwise normal stature, the testis-determining factor or SRY gene may have been removed from the Y chromosome or may be mutated. In other women with Turner syndrome, the karyotype is usually abnormal and shows a frequent 45,X/46,XY mosaicism [12].

There are two patients with short stature. It is reported that fertility can be retained even with the loss of two-thirds of Xp (short arm of X chromosome); thus, if there are genes on Xp for ovarian development, then they must be at Xp11–Xp11.2 [13] (Table 3).

References

1. Damiani D, Guerra G Jr. New definitions and classifications of the intersexual states: in which the Chicago Consensus has contributed to the state of the art? Arq Bras Endocrinol Metabol. 2007;51:1013–7.
2. Fritz MA, Speroff L. Clinical gynecologic endocrinology and infertility. 8th ed. Chapel Hill: Lippincott Williams and Wilkins; 2011. p. 331–60.
3. Park SY, Jameson JL. Minireview: transcriptional regulation of gonadal development and differentiation. Endocrinology. 2005; 146(3):1035–42.
4. Kobayashi A, Behringer RR. Developmental genetics of the female reproductive tract in mammals. Nat Rev Genet. 2003;4: 969–80.
5. Canto P, Soderlund D. Mutations in the desert hedgehog (DHH) gene in patients with 46,XY complete pure gonadal dysgenesis. J Clin Endocrinol Metab. 2004;89(9):4480.
6. Layman LC. Genetic causes of human infertility. Endocrinol Metab Clin North Am. 2003;32(3):549–72.
7. Calvari V, Bertini V, De Grandi A, et al. A new submicroscopic deletion that refines the 9p region for sex reversal. Genomics. 2000;65(3):203–12.
8. Ahmed SF, Cheng A, Dovey L, et al. Phenotypic features, androgen receptor binding, and mutational analysis in 278 clinical cases reported as androgen insensitivity syndrome. J Clin Endocrinol Metab. 2000;85(2):658–65.
9. Boehmer ALM, Bruggenwirth H, Brinkmann O, et al. Genotype versus phenotype in families with androgen insensitivity syndrome. J Clin Endocrinol Metab. 2001;86(9):4151–60.
10. Slowikowska-Hilczer J, Szarras-Czapnik M, Kula K. Testicular pathology in 46,XY dysgenetic male pseudohermaphroditism: an approach to pathogenesis of testis cancer. J Androl. 2001;22(5): 781–92.
11. Josso N, Belville C, di Clemente N, et al. AMH and AMH receptor defects in persistent Müllerian duct syndrome. Hum Reprod Update. 2005;11(4):351–6.
12. Ravel C, Siffroi JP. Y chromosome structural abnormalities and Turner's syndrome. Gynecol Obstet Fertil. 2009;37:511–8.
13. Lachlan KL, Youings S, Costa T, et al. A clinical and molecular study of 26 females with Xp deletions with special emphasis on inherited deletions. Hum Genet. 2006;118:640–51.