Clinical Spectrum of Disorders of Sex Development: 
A Cross-sectional Observational Study

Sheeraz A. Dar, Mudasir Nazir, Roumissa Lone, Duri Sameen, Ikhlas Ahmad, Wasim A. Wani, Bashir A. Charoo
Department of Pediatrics and Neonatology, Sher-I-Kashmir Institute of Medical Sciences Hospital, Srinagar; Department of Community Medicine, Government Medical College, Jammu; Department of Gynecology and Obstetrics, Sher-I-Kashmir Institute of Medical Sciences Hospital, Bemina, Jammu and Kashmir, India

Abstract

Objective: Disorders of sex development (DSD) constitutes a small but difficult and equally important area of endocrinology. It is often a social emergency as the decision regarding sex assignment in these cases is extremely disturbing and difficult to both families and healthcare professionals. Our study was devised to assess the clinical and chromosomal profile of patients with suspected DSD and classify them according to the new DSD consensus document. Subjects and Methods: This study was a cross-sectional observational study carried out in the department of pediatrics of a tertiary care hospital from August 2012 to August 2014. All patients with suspected DSD in the age group of 0–19 years were included. After detailed history and examination, karyotyping, abdominal sonography, and hormonal analysis were done. Additional studies like gonadal biopsy, laparoscopy, and hormone stimulation tests were done in selected cases. Results: About 41 patients were included in the study. The mean age of presentation was 87 months (1 day to 16 years). Only seven (17.3%) patients presented in neonatal period. In total, 25 patients had ambiguous genitalia; 46, XX DSD were diagnosed in 24 (58.5%) patients, 46, XY DSD in 10 (24.4%) patients, and sex chromosome DSD in 7 (17.1%). Congenital adrenal hyperplasia (CAH) was the commonest disease diagnosed in 21 (51.2%) patients. Turner syndrome, Klinefelter syndrome, androgen insensitivity syndrome, 46, XX ovotesticular disorder, and 46, XY gonadal dysgenesis were diagnosed in 3, 3, 4, 3, and 5 patients, respectively. Eleven patients with CAH presented in shock and six had history of sib deaths. Conclusion: 46, XX DSD were the commonest etiological group in our study and CAH was the commonest individual disease. There is a need for educating general public and practitioners regarding DSD to allow early intervention. Moreover, there is a need to introduce routine neonatal screening for CAH in our country.

Keywords: Congenital adrenal hyperplasia, hermaphroditism, intersex, karyotype

Introduction

Disorders of sex development (DSD) are defined as congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical. Considerable confusion and controversy have surrounded the nomenclature of DSD. The Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology consensus modified the nomenclature used to describe atypical sexual differentiation. Instead of using confusing terms, such as “intersex,” “hermaphroditism,” and “sex reversal,” the consensus statement recommended a new taxonomy based on the umbrella term, “DSD.” The disorders were grouped into three broad categories:

1. Sex chromosome DSD (45, XO Turner and variants, 47, XXY Klinefelter and variants, 45, X/46, XY mixed gonadal dysgenesis [MGD] and chromosomal ovotesticular DSD “46, XX/46, XY chimeric type or mosaic type”)
2. 46, XY DSD (disorders of testicular development or disorders in androgen synthesis/action)
3. 46, XX DSD (disorders of ovarian development or fetal androgen excess).

There are limited data on incidence of DSD. The reported incidence varies from 1 in 3,000 to 1 in 5,500. In many cases, it is not possible to identify the sex at first glance.

Address for correspondence: Dr. Mudasir Nazir, Department of Pediatrics and Neonatology, Sher-I-Kashmir Institute of Medical Sciences Hospital, Soura, Srinagar, Jammu and Kashmir - 190 011, India
E-mail: mudasirpaeds@gmail.com

How to cite this article: Dar SA, Nazir M, Lone R, Sameen D, Ahmad I, Wani WA, et al. Clinical spectrum of disorders of sex development: A cross-sectional observational study. Indian J Endocr Metab 2018;22:774-9.
Traditionally, diagnosis in these patients relies on extensive endocrine investigations. With advances in the understanding of genes involved in sexual determination and differentiation, molecular diagnosis is playing an increasingly important role and may even overtake the role of hormonal assessment as the first-line test, with the latter being reserved for assessment of disease severity rather than diagnosis. 

Previous studies from India categorized DSD into three groups: 46, XY DSD; 46, XX DSD; and disorders of gonadal differentiation (including ovotesticular disorders). In the new classification, the DSDs associated with sex chromosomal abnormalities were grouped under a new class of “sex chromosomal DSD,” to differentiate from DSDs with normal chromosome complement (46, XY DSD, 46, XX DSD). Not much data have been published on this problem from our population and hardly any study classifies DSD according to the new classification. Our study was devised to analyze the clinical and chromosomal profile of patients with suspected DSD and to classify these disorders according to the new DSD consensus document.

**Subjects and Methods**

**Study design**

This study was a cross-sectional, observational study carried out in the department of pediatrics of a tertiary care hospital from August 2012 to August 2014. All patients with suspected DSD in the age group of 0–19 years were included. Written informed consent was obtained from the patients and/or parents and the study was approved by the institutional ethical committee. None of the patients/parents refused to participate in the study.

**Clinical definitions**

Criteria that suggested DSD were overt genital ambiguity, apparent female genitalia with an enlarged clitoris, posterior labial fusion, or an inguinal/labial mass; apparent male genitalia with bilateral undescended testes, micropenis, isolated perineal hypospadias, or mild hypospadias with undescended testes; delayed puberty; undervirilization of males; discordance between genital appearance and prenatal karyotype; lymphedema in the newborn period; short stature or learning problems during childhood. Micropenis at birth was defined as stretched penile length of <2.5 cm.

**Data collection and laboratory tests measurement**

Detailed history including detailed pregnancy history, family history of short stature, infertility, sib deaths, and precocious puberty was taken. Thorough physical examination was done in every patient, especially focusing on dysmorphism, anthropometry, and external genitalia examination. Karyotyping and abdominopelvic ultrasound was done in all patients and hormonal analysis including measurement of 17-hydroxyprogesterone, testosterone, cortisol, luteinizing hormone (LH), and follicle stimulating hormone (FSH) in most. Additional studies like fluorescent in situ hybridization for XY chromosomes, human chorionic gonadotrophin (HCG) stimulation tests, urinary steroid analysis, polymerase chain reaction for SRY gene (sex determining region on Y chromosome), and gonadal biopsy were done in selected patients. HCG stimulation test was carried out by giving 1,000 units IM on alternate days for three doses to study the testosterone (T) and dihydrotestosterone (DHT) synthetic response of the gonad. A twofold or more increase in testosterone level was considered a good response and a T to DHT ratio of >20 was considered as suggestive of 5α-reductase deficiency. The data of patients with respect to history, physical examination, baseline, and other disorder-specific investigations was analyzed using GraphPad Instat 3.0 statistical software. In our study, radioimmunoassay methods were used for measurement of 17-hydroxyprogesterone, FSH, LH, serum cortisol, testosterone, and DHT levels. Abdomino-pelvic ultrasonography was done by a consultant radiologist. For karyotyping, white blood cells from patient’s venous blood were obtained and were stained with Giemsa stain that was applied after cells were arrested during cell division by a solution of colchicine.

**Results**

In total, 41 patients with DSD were included in the study. Etiological categorization and age at presentation of patients are given in Table 1. Sex chromosome DSD was diagnosed in 7 (17.1%) [Table 2], 46, XY DSD in 10 (24.4%) [Table 3], and 46XX DSD in 24 (58.5%) patients [Table 4]. Congenital adrenal hyperplasia (CAH) was the commonest disease diagnosed in 21 (51.2%) patients. About 25 patients had ambiguous genitalia; 21 among them belonged to CAH group, 2 to ovotesticular disorders, and 1 each to 5α-reductase deficiency syndrome (5α-RDS) and complete gonadal dysgenesis. Comparison of patient parameters between previous studies and this study is given in Table 5.

**Discussion**

Overall, 51% patients in our study presented after 1 year of age, and only 13.7% presented in neonatal period. These findings are consistent with the results of other studies from India. However, studies from developed countries reported that most patients presented in neonatal period, and the treatments (including surgical) were instituted before 6 months of age in most cases, which is often delayed in our setup. Presentation at later age for so many patients points to the taboos and ignorance existing in our society with regard to DSD and reflects the need to sensitize both parents and doctors about the importance of early diagnosis of these disorders. The new classification includes chromosomal disorders like Turner’s syndrome, in which both chromosomal and gonadal sex are abnormal despite normal external genitalia. These disorders may present later with short stature or delayed puberty, as is seen in our study, increasing the average age of presentation.
Dar, et al.: Clinical spectrum of disorders of sexual development

Table 1: Distribution of patients with DSD

| Type                                  | Number (%) | Age at presentation, median (range) | Ambiguous genitalia |
|---------------------------------------|------------|-------------------------------------|---------------------|
| Sex chromosome DSD                   | 7 (17.1%)  | 4.5 years (5 days-16 years)         | 0                   |
| 45, XO                                | 3 (7.3%)   | 0                                   | 0                   |
| 47, XXY                               | 3 (7.3%)   | 14 (5-15) years                     | 0                   |
| 46, XX/46, XY ovotesticular disorder  | 1 (2.4%)   | 4 years                             | 0                   |
| 46, XY DSD                            | 10 (24%)   |                                     | 2                   |
| CAIS                                  | 4 (9.7%)   | 14 (5-15) years                     | 0                   |
| 46, XY gonadal dysgenesis            | 5 (12%)    | 15 (1-16) years                     | 1                   |
| 5α-Reductase deficiency Syndrome     | 1 (2.4%)   | 8 years                             | 1                   |
| 46, XX DSD                            | 24 (58.5%) | 36 (1-90) days                      | 23                  |
| CAH (salt-losing)                     | 16 (39.0%) |                                     | 16                  |
| CAH (simple virilizing)              | 5 (12.2%)  | 2 days (1 day-5 years)              | 5                   |
| 46, XX ovotesticular disorder        | 3 (7.3%)   | 4 (3-12) years                      | 2                   |

Table 2: Categorization; clinical and laboratory parameters of patients with sex chromosome DSD

| Diagnosis                        | Age   | Presenting complaint | Clinical features                                                                 | Laboratory parameters                              | Karyotype | Imaging                                      |
|----------------------------------|-------|----------------------|----------------------------------------------------------------------------------|----------------------------------------------------|-----------|-----------------------------------------------|
| Turner syndrome                  | 5 days| Sepsis               | Webbing of neck, low hair line, wide spaced nipples, edema of hands and intrauterine growth retardation | High FSH and LH                                     | 45, XO    | Prepubertal Mullerian ducts and streak ovaries |
| Turner syndrome                  | 16 yrs| primary amenorrhea   | Short-stature                                                                     | Elevated FSH and LH                                 | 45, XO    | Pre-pubertal Mullerian ducts and streak ovaries |
| Turner syndrome                  | 4.5 yrs| short stature        | Webbed neck                                                                       | Elevated FSH and LH                                 | 45, XO    | pre pubertal Mullerian ducts and streak ovaries , coarctation of aorta |
| Klinefelter syndrome             | 14 yrs| delayed puberty      | Gynecomastia, palpable prepubertal size intra scrotal testes                       | Low testosterone and high FSH and LH                 | 46, XXY   | Normal Wolffian structures                    |
| Klinefelter syndrome             | 15 yrs| delayed puberty      | Gynecomastia, palpable prepubertal size intra scrotal testes                       | Low testosterone and high FSH and LH                 | 46, XXY   | Normal Wolffian structures                    |
| Klinefelter syndrome             | 7 yrs | delayed speech/social development | Palpable prepubertal size intra scrotal testes                                    | Low testosterone and high FSH and LH                 | 46, XXY   | Normal Wolffian structures                    |
| 46, XX/XY ovotesticular disorder  | 4 yrs | Ambiguous genitalia | Clitoromegaly, bilateral inguinal gonads                                           | 46, XX/XY mosaicism (on FISH)                       |           | Intra-abdominal uterus and prostate           |

FSH: Follicle stimulating hormone, LH: Luteinizing hormone, FISH: Fluorescent in situ hybridization

On the contrary, most patients in the study of Joshi et al. presented early. However, in a study from north India, 70% patients presented after 5 years.

In our study, 61% had ambiguous genitalia. Patients with ambiguous genitalia are more likely to be diagnosed and treated early; taboos associated with these patients in conservative societies like ours may still delay presentation till complications set in. Patients with 46, XY gonadal dysgenesis syndrome in our study presented at 14–16 years with delayed puberty. Patients with CAH and ambiguous genitalia, on the other hand, were evaluated even on first day of their life.

46, XX DSD were the most common form of DSD in our study, with CAH as the most common underlying diagnosis. These observations were consistent with the results of relevant Indian and foreign studies. Further, we observed that most (51%) of patients with CAH had underlying deficiency of 21-hydroxylase enzyme. These results were similar to other studies. Gupta et al. reported that over 90% of CAH have the 21-hydroxylase deficiency, with build up of 17-OHP, a by-product prior to the block. In a study by Jaruratanasirikul as well, CAH was the most common cause of DSD if only patients with genital ambiguity were considered. The age at presentation in these patients varied from 1 day for those presenting with genital ambiguity to 5 years for those presenting with precocious puberty. The presentation of a high proportion of CAH patients with shock (52.4%) and unexplained sib death (33%) emphasizes the need to include routine neonatal screening for 21-hydroxylase deficiency on national level in our country.

In our study, 24.39% patients had 46, XY DSD. Out of these, four had complete androgen insensitivity syndrome, one
had 5α-reductase deficiency, and five had 46, XY gonadal dysgenesis syndrome. Prevalence of 46, XY DSD is less than reported in other studies from India, however consistent with studies from outside of India. The difference can be explained by inclusion of chromosomal DSD in our study as per new classification. Besides, some have included male CAH patients in their study, but since they present with precocity and not genital ambiguity, we have not included them in DSD. One of the patients with complete gonadal dysgenesis had normal Mullerian structures with completely female external genitalia except for mild clitoromegaly. Genital ambiguity is an unexpected finding in complete gonadal dysgenesis, though clitoromegaly can be present. The gonads of this patient consisted of almost totally undifferentiated streaks. Partial gonadal dysgenesis (varying degrees of ambiguous external genitalia with or without Müllerian structures) and gonadal agenesis or embryonic testicular regression syndrome (slightly ambiguous external genitalia without Müllerian structures) were close differentials.

| Table 3: Categorization; clinical and laboratory parameters of patients with sex chromosome DSD 46, XY DSD |
|---|---|---|---|---|---|
| Diagnosis | Age (years) | Presenting complaint | Clinical features | Laboratory parameters | Imaging | Karyotype/gonadal biopsy |
| 46, XY GD | 14 | Delayed puberty | Small breasts, no pubic hair | high FSH and LH levels | Bilateral intra-abdominal streak gonads | 46, XY |
| 46, XY GD | 1 | - | Clitoromegaly | - | Streak intra-abdominal gonads and uterus | Sparse and disordered 5α-Reductase deficiency syndrome—embryonic testicular regression syndrome 
46, XY |
| 46, XY GD | 15 | Delayed Puberty | - | high FSH and LH levels | Uterus and bilateral intra-abdominal streak gonads | 46, XY |
| 46, XY GD | 16 | Delayed puberty | - | high FSH and LH levels | Uterus and bilateral intra-abdominal streak gonads | 46, XY |
| CAIS | 15 | Primary amenorrhea | Left inguinal testis, scanty pubic and axillary hair | normal to high LH and normal FSH, HCG stimulation test positive | Intra-abdominal gonads but no Mullerian structures | 46, XY |
| CAIS | 16 | Primary amenorrhea | No pubic or axillary hair | HCG stimulation test was positive | Intra-abdominal gonads but no Mullerian structures | 46, XY |
| CAIS | 14 | Primary amenorrhea | Right inguinal testis | HCG stimulation test was positive | Intra-abdominal gonads but no Mullerian structures | 46, XY |
| CAIS | 17 | Primary amenorrhea | No pubic or axillary hair | normal to high LH and normal FSH levels, HCG stimulation test positive | Intra-abdominal gonads but no Mullerian structures | 46, XY |
| 5αRDS | 8 | Right inguinal hernia | Clitoromegaly, some posterior labial fusion | positive HCG stimulation test and high T:DHT ratio | Mullerian structures absent | 46, XY |

GD: Gonadal dysgenesis, CAIS: Complete androgen insensitivity syndrome, 5α-RDS: 5α-reductase deficiency syndrome, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, HCG: Human chorionic gonadotrophin

| Table 4: Clinical presentation and laboratory parameters of patients with 46, XX DSD |
|---|---|---|---|
| CAH (salt-losing) | CAH (simple virilizing) | 46, XX ovotesticular disorder |
| Number (%), n=24 | 16 (66.7) | 5 (20.8) | 3 (12.5) |
| Age at presentation | 36 (1-90) d | 2 d (1d-5y) | 3 (3-12) y |
| Clinical presentation | Ambiguous genitalia (16), refusal of feeds (13), lethargy (13), dehydration (11), recurrent vomiting (11), shock (11), weight loss (6), hypoglycemia (3), sib deaths (6), hypoglycemia (3) | Ambiguous genitalia (5), growth spurt (2), undescended testis (1), sib death (1) | Undescended testis (3), hypospadias (2) |
| History of consanguinity, n (%) | 8 (50) | 2 (40) | 3 (100) |
| Investigations | Hyponatremia (14), hyperkalemia (9), hypoglycemia (3), raised 17-hydroxyprogesterone (16), high testosterone (14), adrenal hyperplasia on ultrasonography (1) | Raised 17-hydroxyprogesterone (5), high testosterone levels (3) | uterus and intra-abdominal testes (3), both ovarian and testicular tissue within the gonads on biopsy (3), 46, XX karyotype (3) |

CAH: Congenital adrenal hyperplasia, DSD: Disorders of sex development

Indian Journal of Endocrinology and Metabolism ¦ Volume 22 ¦ Issue 6 ¦ November-December 2018
In our study, 17.1% had sex chromosome DSD. Sex chromosome DSD were the most common cause of DSD in the study Jaruratanasirikul et al. However, in other studies they were not that common. Other studies from India have not included sex chromosome DSD. As two out of the three patients diagnosed with turner syndrome presented with short stature, all female children presenting with short stature and primary amenorrhea, with or without phenotypic features of turners syndrome, should be evaluated for turner syndrome. A patient with Klinefelter syndrome presented with delayed speech and social development, emphasizing the need to consider this diagnosis when the cause of delayed speech is not obvious.

Three patients in our study had 46, XX ovotesticular disorder. The level of genital ambiguity in these patients was variable. In patients with genital ambiguity who have 46, XX chromosome, an important physical finding which is suggestive of presence of testis and can lead to the diagnosis of ovotesticular DSD, is a palpable gonad at the inguinal area. Clinical features that can differentiate CAH from gonadal DSD (MGD and ovotesticular DSD) are progressive virilization and testosterone level. In patients with CAH, virilization is progressive with time and the testosterone level is markedly elevated for age, whereas in patients with gonadal DSD, the virilization is nonprogressive and the testosterone level is normal for age. Further, presence of Mullerian and Wolffian ducts, along with ovary and testis or ovotestes, found through laparoscopic examination, suggests the diagnosis of ovotesticular DSD.

Our study had many limitations. This was a cross-sectional study and the long-term follow-up of patients was not available. The study sample size was small owing to the rarity of the disorder and shorter duration of study. Further, molecular analysis and genetic mutations for specific disorders were not performed. However, there are strengths of this study as well. This is the first study evaluating the etiological diagnoses of DSD according to the new classification from our place and we believe that it provides some interesting data.

### Conclusion

46, XX DSD were the commonest etiological group in our study and CAH the commonest individual disease. There is a need for educating general public and practitioners regarding DSD, to allow early intervention. Since CAH patients presented during infancy with adrenal crisis and shock and many had history of unexplained sib deaths in early infancy, it appears desirable to introduce routine neonatal screening for 21-hydroxylase deficiency in our country.
Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Lee PA, Houk CP, Ahmed SF, Hughes IA. International Consensus Conference on Intersex organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. Consensus statement on management of intersex disorders. International Consensus Conference on Intersex. Pediatrics 2006;118:488-500.
2. Hughes IA. Disorders of sex development: A new definition and classification. Best Pract Res Clin Endocrinol Metab 2008;22:119-34.
3. Blackless M, Charuvastra A, Derryck A, Fausto-Sterling A, Lauzanne K, Lee E. How sexually dimorphic are we? Review and synthesis. Am J Hum Biol 2000;12:151-66.
4. Sax L. How common is intersex? A response to Anne Fausto-Sterling. J Sex Res 2002;39:174-8.
5. Melton L. New perspectives on the management of intersex. Lancet 2001;357:2110.
6. Ono M, Harley VR. Disorders of sex development: New genes, new concepts. Nat Rev Endocrinol 2013;9:79-91.
7. Arboleda VA, Lee H, Sanchez FJ, Delot EC, Sandberg DE, Grody W, et al. Targeted massively parallel sequencing provides comprehensive genetic diagnosis for patients with disorders of sex development. Clin Genet 2013;83:35-43.
8. Kulkarni KP, Panigrahi I, Das R, Kaur S, Marwaha RK. Pediatric disorders of sex development. Indian J Pediatr 2009;76:956-8.
9. Halder A. Disorder of sex development: Spectrum of disorders in a referral tertiary care hospital in North India. Global J Hum Genet Gene Ther 2013;1:77-89.
10. Joshi RR, Rao S, Desai M. Etiology and clinical profile of ambiguous genitalia - An overview of 10 years experience. Indian Pediatr 2006;43:974-9.
11. Ammini AC, Gupta R, Kapoor A, Karak A, Kriplani A, Gupta DK, et al. Etiology, clinical profile, gender identity and long term follow up of patients with ambiguous genitalia in India. J Pediatr Endocrinol Metab 2002;15:423-30.
12. Rajendran R, Hariharan S. Profile of intersex children in South India. Indian Pediatr 1995;32:666-71.
13. Gupta DK, Menon PSN. Ambiguous genitalia: An Indian perspective. Indian J Pediatr 1997;64:189-94.
14. Garry LW, Jeffrey DZ. Evaluation of a child with ambiguous genitalia: A practical guide to diagnosis and management. In: Meena PD, Vijayalaxmi B, Menon PSN, editors. Pediatric endocrine disorders. New Delhi, India: Orient Longman Ltd.; 2001. p. 257-76.
15. Walia R, Singla M, Vaiphei K, Kumar S, Bhansali A. Disorders of sex development: A study of 194 cases. Endocr Connect 2018;7:364-71.
16. Al-Agha AE, Thomsett MJ, Batch JA. The child of uncertain sex: 17 years of experience. J Paediatr Child Health 2001;37:348-51.
17. Thyen U, Lanz K, Holterhus PM, Hiort O. Epidemiology and initial management of ambiguous genitalia at birth in Germany. Horm Res 2006;66:195-203.
18. Nimkam S, Likitmoskul S, Sangcecharoenkit P, Pathomvanich A, Sawathiparnich P, Wacharasindhu S, et al. Ambiguous genitalia: An overview of 22 years experience and the diagnostic approach in the Pediatric department, Siriraj Hospital. J Med Assoc Thai 2002;85:496-505.
19. Jaruratanasirikul S, Engchaun V. Management of children with disorders of sex development: 20-year experience in southern Thailand. World J Pediatr 2014;10:168-74.
20. Mendonca BB, Domenice S, Arnhold IJ, Costa EM. 46,XY disorders of sex development (DSD). Clin Endocrinol (Oxf) 2009;70:173-87.
21. Al-Muttair A, Iqbal MA, Sakati N, Ashwal A. Cytogenetics and etiology of ambiguous genitalia in 120 pediatric patients. Ann Saudi Med 2004;24:368-72.