Original Article

Disorders of Sex Development: A 10 Years Experience with 73 Cases from the Kashmir Valley

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Abstract

Purpose: To present the clinical data, investigative profile, and management of patients with disorders of sex development (DSD) from the endocrine unit of a tertiary care university hospital. **Materials and Methods:** This retrospective study included 73 cases of DSD, evaluated and managed at Department of Endocrinology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Kashmir, over a period of 10 years from September 2008 to August 2018. **Results:** Twenty-nine patients (39.7%) had 46 XY DSD and twenty-nine patients (39.7%) had 46 XX. Sex chromosome DSD was diagnosed in 15 (20.5%) patients. Of 29 patients with 46 XY DSD, 17 (58.6%) had 5α-reductase type-2 deficiency (5α-RD) and 6 (20.7%) had complete androgen insensitivity syndrome. In our patients with 5α-RD, the history of consanguinity was documented in nine (52.9%) patients. Two patients had testosterone biosynthetic defect and one patient had partial androgen insensitivity syndrome. Of 29 patients with 46 XX DSD, 16 (55.1%) had congenital adrenal hyperplasia (CAH). Of 15 patients with sex chromosome DSD, 7 patients had Turner's syndrome, 7 had Klinefelter's syndrome, and 1 patient had mixed gonadal dysgenesis. **Conclusion:** In our study, equal number of patients had 46 XY DSD and 46 XX DSD. We are for the first time reporting from India that the most common cause of 46 XY DSD is 5α-RD, whereas CAH is the most common cause of 46 XX DSD as reported previously.

Keywords: 5α-reductase type-2 deficiency, ambiguous genitalia, congenital adrenal hyperplasia, disorders of sex development

Introduction

Disorders of sex development (DSD) are defined as congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical.[1] In 2006, the Lawson Wilkins Pediatric Endocrine Society (LWPES) and the European Society for Pediatric Endocrinology (ESPE) published a consensus statement on the management of intersex disorders and proposed the umbrella term "DSD" instead of terms like "intersex," pseudohermaphroditism, "hermaphroditism," and "sex reversal," which are often perceived as pejorative by patients and can be confusing to both health professionals and parents.[2] The incidence of DSD is 1:4500 to 1:5000 live births.[3,4] The birth of a child with a DSD is a social emergency as the decision-making in relation to sex assignment has been perceived as extremely disturbing and difficult to both families and health care professionals.[5] DSD can also be a medical emergency as patient may present with life-threatening adrenal crisis. In this retrospective study, we present the clinical data, investigative profile, and management of DSD patients from the endocrine unit of a tertiary care university hospital.

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Materials and Methods

This retrospective study included 73 patients with DSD evaluated and managed at our center over a period of 10 years from September 2008 to August 2018. The clinical evaluation included detailed history and physical examination. History focused on family history (consanguinity, three-generation pedigree, genital ambiguity/DSD, and sibling deaths), age at presentation, sex of rearing, and features suggestive of salt-wasting crisis. Physical examination focused on the presence of hyperpigmentation (in particular genital

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skin, nipples, and palmar creases), blood pressure, and anthropometry. Local genital examination focused on phallic length, palpable gonads and their location, position of urethral opening or presence of common urogenital sinus, labioscrotal folds with degree of fusion, and rugosity of scrotum. In our patient cohort, features which suggested the diagnosis of DSD included overt genital ambiguity, apparent male genitalia with nonpalpable testes, micropenis, isolated perineal, or penoscrotal hypospadias, apparent female genitalia with clitoromegaly, posterior labial fusion, and inguinal/labial mass, family history of DSD, and genital/karyotype discordance.[2] Baseline investigations included complete blood count, kidney function test, venous blood gases, and serum sodium and potassium. All our patients had a karyogram; a minimum of 20 metaphase chromosomes were examined in each case. In addition, all our patients underwent abdominopelvic sonography and/or MRI for localization of gonads and the presence of mullerian structures.

All patients with 46 XY DSD baring those with complete androgen insensitivity syndrome (CAIS) were given a human chorionic gonadotropin (hCG) stimulation test. This was performed by giving intramuscular injection of 1000–1500 units of hCG for 3 consecutive days and taking blood samples before the first injection for serum testosterone (T) and 24 h after the third injection for T, dihydrotestosterone (DHT), and androstenedione (A). A twofold or greater increase in T level was considered a good response (functional testis). A testosterone to DHT ratio of >20 was considered as suggestive of 5α-reductase type-2 deficiency (5α-RD).[6] Androgen biosynthetic defect, 17β-hydroxysteroid dehydrogenase type-3 (17β-HSD3) deficiency was diagnosed in the presence of low T and high luteinizing hormone (LH) and follicle-stimulating hormone (FSH) values during pubertal and post-pubertal period with T/A ratio <0.8 in response to hCG stimulation.[6] The diagnosis of partial androgen insensitivity syndrome (PAIS) was based on the presence of genital ambiguity and high basal LH, FSH, and T on either side of the "window period." In all patients with 46 XX karyotype, the evaluation included estimation of 8 AM or random serum cortisol, 17-hydroxyprogesterone (17-OHP), testosterone, LH, and FSH. Congenital adrenal hyperplasia (CAH) was diagnosed if unstimulated and/or stimulated (after intravenous injection of 250 μg of tetracosactin) 17-OHP level was >10,000 ng/dl.[7]

A few of our patients underwent laparoscopy and/or gonadal biopsy to identify the internal structures and ascertain gonadal morphology.

All hormones, except 17-OHP, were measured by DXI 800, Beckman Coulter Chemiluminescence Random-access analyzer (Brea/CA) following the manufacturer's protocol; 17-OHP was measured by ELISA. The study was approved by institute ethics committee.

Results

In total, 73 patients with DSD were included in the study; these included 29 (39.7%) with 46 XY DSD, 29 (39.7%) with 46 XX DSD, and 15 (20.5%) with sex chromosome DSD. The various etiologies of DSD are shown in Table 1. Age of presentation in our study varied from birth to 35 years. The majority of patients (87.9%) presented before 5 years of age.

46 XX DSD

Twenty-nine patients were classified as 46 XX DSD [Table 1]. CAH was the most common diagnosis present in 24 patients (82.8%). Sixteen (66.6%) patients with CAH had simple virilizing CAH. The age of presentation of simple virilizing CAH varied from birth to 28 years. Eight (33.3%) patients had salt wasting CAH and presented with crisis between 1 and 5 weeks of age. Due to lack of facilities, we could not perform genetic analysis of our CAH patients. One patient with simple virilizing CAH, reared as male, was referred to urology department at the age of 28 years with cyclical hematuria which in fact represented regular menstrual cycles. Two of our CAH patients had possible 11β-hydroxylase deficiency on the basis of genital ambiguity, hypertension, and hypokalemia. Over one third (37.5%) of CAH patients had Prader stage 2, while 8.3%, 25%, 16.7%, and 12.5% had Prader stage 1, 3, 4, and 5 virilization of external genitalia, respectively. All CAH patients, except the two with possible 11β-hydroxylase deficiency, were treated with hydrocortisone (10–15 mg/m^2/day) and fludrocortisone (50–100 μg/day). The latter were treated with hydrocortisone and spironolactone.

Two patients with mullerian agenesis presented with primary amenorrhea and well-developed secondary sexual characters. One of them also had single kidney and was diagnosed as Mayer Rokitansky Kuster Hauser syndrome. Two patients diagnosed as gonadal dysgenesis presented with failure of onset of puberty. Both patients had eunuchoidal body proportions, female external genitalia, and mullerian structures. Investigations revealed high gonadotropin levels and hypoestrogenemia (serum estradiol <20 pg/ml). Bilateral gonadectomy was performed and histology was consistent with streak gonads. Estrogen replacement therapy was initiated. One patient diagnosed as ovotesticular DSD (Karyotype 46 XX) reared as male presented at 3 months of age with asymmetric labioscrotal fold (less developed on left side) with bilateral palpable gonads in labioscrotal folds. Laparotomy and histopathology revealed rudimentary uterine structure, testis on right side, and ovotestis on left side.

46 XY DSD

Twenty-nine patients were classified as 46 XY DSD [Table 1]. The most common etiology was 5α-RD, diagnosed in 17 (58.6%) patients. Most of these patients presented in infancy. Perineal or penoscrotal hypospadias was present in 15, bifid scrotum in 8, and micropenis in 8 patients [Table 2]. Out of 17 patients with 5α-RD, 13 (76.4%) had descended testes, 5 in inguinal canal, 4 in labioscrotal folds, and 4 in scrotum. The ratio of Testosterone/DHT ranged from 21.2 to 75.4. History of consanguinity was documented in 9 patients and 3 patients were initially reared as females. Uretheroplasty had been performed in 15 patients and 4 patients had undergone orchidopexy.

Table 1: Various etiologies of DSD

Sex Chromosomal DSD *n*=15 (20.6%)	*n* (%)	46 XX DSD *n*=29 (39.7%)	*n* (%)	46XY DSD *n*=29 (39.7%)	*n* (%)
47XXY (Klinefelter's Syndrome)	7 (46.7)	CAH	24 (82.7)	5α-RD	17 (58.6)
45X0 (Turner's Syndrome)	5 (33.3)	Gonadal dysgenesis	2 (6.8)	CAIS	6 (20.7)
45X0/46XX (MosaicTurner's Syndrome)	2 (13.3)	Mullerian agenesis/hypoplasia	2 (6.8)	17β-HSD3 deficiency	2 (6.9)
45X0/46XY (Mixed gonadal dysgenesis)	1 (6.7)	Ovotesticular	1 (3.4)	PAIS	1 (3.4)
				Partial Gonadal Dysgenesis	1 (3.4)
				Vanishing Testis Syndrome	1 (3.4)
				CAH	1 (3.4)

CAH: Congenital adrenal hyperplasia; 5α-RD: 5α-reductase type-2 deficiency; CAIS: Complete androgen. Insensitivity syndrome; 17β-HSD3: 17β-hydroxysteroid dehydrogenase type-3; PAIS: Partial androgen insensitivity syndrome

Table 2: Clinical profile of patients with 5α-reductase type-2 deficiency

Age at presentation	Consanguity	Clinical phenotype	HCG stimultated T/DHT ratio
2 months	Yes	Penoscrotal hypospadias	31.9
1 month	Yes	Micropenis; Penoscrotal hypospadias	21.2
Birth	Yes	Bifid scrotum; Perineal hypospadias	36.8
3 months	Yes	Bifid scrotum; Penoscrotal hypospadias	30.2
4 years	No	Bifid scrotum; Penoscrotal hypospadias	26.0
2 day	No	Bifid scrotum; Perineal hypospadias	42.0
Birth	No	Penoscrotal hypospadias	52.8
1 month	Yes	Micropenis; Bifid scrotum	71.0
13 years	Yes	Penoscrotal hypospadias	65.2
3 months	Yes	Micropenis; Penoscrotal hypospadias	75.4
Birth	No	Penoscrotal hypospadias	47.4
Birth	No	Micropenis; Penoscrotal hypospadias	54.0
13 years	No	Bifid scrotum; Perineal hypospadias	25.0
1 year and 6 months	Yes	Micropenis; Penoscrotal hypospadias	33.6
3 months	No	Micropenis; Perineal hypospadias; Bifid scrotum	55.5
2 years	No	Micropenis; Penoscrotal hypospadias	27.3
Birth	Yes	Micropenis; Bifid scrotum	29.8

HCG: Human chorionic gonadotropin; T: Testosterone; DHT: Dihydrotestosterone

CAIS was diagnosed in six patients; three presented in childhood with inguinal hernia and three at adolescence with primary amenorrhea. History of consanguinity was present in 3 patients and family history of infertility in maternal aunts in 2 patients. All had female external genitalia and gonads palpable in inguinal region. Bilateral gonadectomy had been performed in 4 patients at completion of puberty. Following gonadectomy, these patients were started on estrogen replacement therapy. Two patients were diagnosed to have 17β-HSD3 deficiency and both presented at puberty. One patient, reared as male, presented at 14 years of age with bifid scrotum and hypospadias; the hCG stimulated T/A ratio was 0.7. Uretheroplasty with orchidopexy was performed. The other patient was reared as female till the age of 13 years when progressive virilization occurred and gender assignment was changed to male. The hCG stimulated T/A ratio was 0.5. Both the patients had low basal T and high LH and FSH. Urethroplasty was performed and testosterone replacement was started.

PAIS was diagnosed in one patient who presented to us at the age of 16 years with gynecomastia. He was born with ambiguous genitalia but parents surprisingly did not seek medical attention till 13 years of age at which time he underwent two-stage uretheroplasty for penoscrotal hypospadias. Clinical examination revealed gynecomastia, stage IV pubic hair, testicular volume of 15 ml on either side, and micropenis. Biochemical evaluation revealed markedly high testosterone (1528 ng/dl), elevated LH (17.5 IU/L), FSH of 7.54 IU/L, and elevated estradiol (37 pg/ml). The patient refused reduction mammoplasty and was started on tamoxifen 20 mg per day. One patient diagnosed with vanishing testis syndrome presented at age of 1 year with unambiguous male genitalia, nonpalpable gonads, and perineal hypospadias. Basal T was <10 ng/dl, serum gonadotropin levels were elevated, AMH levels were 0.02 ng/ml, and testosterone response to hCG was flat. MRI pelvis and laparoscopy failed to locate gonads.

One of our patients with 46 XY DSD had salt wasting CAH with genital ambiguity. He presented to a pediatrician at the age of 1 week with seizures related to hypoglycemia and hyponatremia and was treated as adrenal crisis and started on hydrocortisone and fludrocortisone treatment. The child

was referred to us at age of 18 months for further evaluation and management. Examination revealed micropenis, bilateral testis in labioscrotal folds without any skeletal anomalies. Evaluation revealed 8 AM cortisol of 7.11 μg/dl, 17–OHP of 11,234 ng/dl, and testosterone of 202 ng/dl. There was the history of sibling death at 2 days of age. The clinical and investigative profile of this patient was strongly suggestive of either 3β-hydroxysteroid dehydrogenase type-2 deficiency or P450 oxidoreductase deficiency as these are the only two etiologies of CAH which lead to ambiguous genitalia in a genetic male.

Sex chromosome DSD

Of the 15 patients with sex chromosome DSD, 7 patients had Turner's syndrome (5 classical and 2 mosaic), 7 had Klinefelter's syndrome, and 1 had mixed gonadal dysgenesis (MGD). All patients with Turner's syndrome presented after 12 years of age with three presenting as primary amenorrhea, three with short stature, and one referred in view of clinical stigmata of Turner's syndrome. Patients with Klinefelter's syndrome presented at a mean age of 31 years with primary infertility and azoospermia. Interestingly, one patient had history of unilateral orchidopexy in childhood and no evaluation for DSD was done at that time. The lone patient with MGD presented at the age of 14 years with perineal hypospadias, micropenis, and bifid empty scrotum. Karyotype revealed 45XO/46XY—the typical karyotype of MGD. MRI revealed uterus and T2 hyperintense structure along left internal iliac vessels (testis). Laparotomy along with hysterectomy and left gonadectomy was done; right gonad could not be identified. Biopsy of left testis revealed dysgenetic testis. He was started on monthly injectable testosterone replacement.

Discussion

Contrary to all the previous Indian studies[8-12] that reported a significant preponderance of 46 XY DSD, our study revealed an equal representation of 46 XY and 46 XX DSD. Again unlike the previous Indian studies[8,10] that revealed the androgen insensitivity syndromes to be the commonest etiology of 46 XY DSD, we found 5α-RD to be the most common etiology of 46 XY DSD. In a study from northern India, only two of the 45 patients with 46 XY DSD patients had 5α-RD.[12] The cluster of cases of 5α-RD have been reported from certain geographic regions like Dominican Republic, New Guinea, South Lebanon, Turkey, and Mexico.[13,14] As consanguinity is common in 5α-RD, we documented consanguinity in 52.9% of our 5α-RD patients. In an Indian study involving 40 cases of 46 XY DSD, history of consanguinity was obtained in only 1 patient.[9] There are two reasons for 5α-RD as the most common etiology of 46 XY DSD in our study. First, the high rates of consanguinity in our population (from socioreligious reasons). Second, most of the earlier Indian studies have not estimated DHT; therefore, there is a probability that 5α-RD may have remained under-diagnosed.[9,11,12,15,16] Early diagnosis is mandatory in these patients to prevent psychological suffering which occurs when a female-to-male gender identity switch is requested, as seen in three patients in our study. The clinical features in our 5α-RD patients included perineal or penoscrotal hypospadias, bifid scrotum, and micropenis. Majority of our patients with 5α-RD had descended gonads; this highlights the primacy of testosterone and not DHT in testicular descent.

17β-HSD3 deficiency is an autosomal recessive disorder characterized by ambiguous genitalia and marked virilization at the time of puberty due to conversion of androstenedione to testosterone by extraglandular 17β-HSD5.[17] Due to marked virilization at puberty, female-to-male gender reassignment is common. In our study, two patients had 17β-HSD3 deficiency, and in one patient reared as female, the sex of rearing was changed to male at puberty.

CAH is the most common cause for DSD worldwide. It is usually diagnosed at birth during routine newborn screening.[7] However, due to lack of newborn screening program in developing countries, patients with CAH are usually diagnosed late when they present with salt-wasting and/or virilization.[2] Eight (33.3%) of our CAH patients had salt wasting, which is a life-threatening condition. One should always consider this possibility in a child with vomiting and failure to thrive, especially in setting like ours where screening for CAH is not routinely done. Salt wasting CAH, especially in boys, gets misdiagnosed as congenital pyloric stenosis or neonatal sepsis. Ten (42%) of our CAH patients who were genetically female were initially reared as male, and six of these were ultimately reared as females. Severity of virilization of genitalia and parental insistence for rearing as a male significantly influenced the choice of sex of rearing. In all these patients, gender identity and role were male, strengthening the hypothesis that prenatal exposure to androgens is important in masculinizing the brain.[18] As fertility can be restored in all patients of CAH, it is recommended to rear all of them as females. Delayed presentation and bias toward male sex of rearing are peculiar to our setting as has been highlighted by other Indian studies.[8-12,15,16] All four CAH patients ultimately reared as males underwent laparotomy and removal of female internal genital organs and were started on testosterone replacement.

The strengths of the study are large number of subjects and detailed clinical profile with biochemical confirmation. Limitations of the study include lack of genetic workup and retrospective nature.

Conclusion

In conclusion, we are first time reporting from India that the most common cause of 46 XY DSD is 5α-RD, whereas CAH is the most common cause of 46 XX DSD as reported previously. Delayed diagnosis and delayed referral are a common feature.

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Conflicts of interest

There are no conflicts of interest.

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