Case Reports

Application of management algorithm to a series of patients of disorders of sexual differentiation from Southern India: A case series

Ankur Rawat[1], **Utpal SD**[2], ***Sudhanshu Tiwari**[3], **Deepu V Joy**[4], **Apoorv Saxena**[5]

Sri Lanka Journal of Child Health, 2024: **53**(1): 63-66
DOI: https://doi.org/10.4038/sljch.v53i1.10603
(Key words: Disorders of sexual differentiation, Ambiguous genitalia, Aromatase deficiency, Ovo-testicular DSD, Congenital adrenal hyperplasia, Testicular dysgenesis, Androgen insensitivity syndrome)

Introduction

Disorders of sexual development (DSD) are congenital conditions characterized by atypical development of anatomical, gonadal or chromosomal sex[1,2,3]. DSDs have an incidence of 1:4500 to 1:5000 live births[1-10]. They can be classified as gonadal dysgenesis, under-virilisation of 46, XY individuals (female phenotype with male genotype) and over-virilization of 46, XX individuals (male phenotype with female genotype)[1]. As per newer classification, DSD can be divided into 3 major groups, sex chromosome DSD, 46, XX DSD and 46, XY DSD[4,5].

A neonate presenting with ambiguous genitalia is a social emergency as decision-making related to gender assignment is difficult for family and health professionals[4]. Clinical diagnosis, surgical/ medical treatment options, and need for hormone therapy have to be considered along with wishes of family and prevailing cultural practices. These may have long-term effects on gender identity, fertility and tumorigenesis affecting quality of life in later years[5]. Thus, management of DSD requires a coordinated approach by a team comprising paediatrician, endocrinologist, paediatric surgeon, a radiologist together with a skilled laboratory set-up[6]. Figure 1 is a diagnostic algorithm for DSD.

Case series

There were 7 cases of ambiguous genitalia presenting to our centre from January 2019 to June 2020. Diagnosis was based on published criteria for diagnosing DSD[2,4,5]. Each patient's data, clinical features and laboratory findings were analysed by the specialist team as per algorithm shown in Figure 1.

[1]*Dept. of Paediatrics, Military Hospital Mathura, India,*
[2]*Dept. of Paediatrics, Military Hospital Hissar, India,*
[3]*Dept. of Paediatrics, Military Hospital Jaipur, India,*
[4]*Dept. of Paediatrics, Military Hospital Sagour, India,*
[5]*Dept, of Paediatrics, Military Hospital Ahmedabad, India*
**Correspondence:* drsudhanshuafmc@gmail.com
https://orcid.org/0000-0002-7227-9747

(Received on 25 May 2023: Accepted after revision on 21 July 2023)
The authors declare that there are no conflicts of interest
Personal funding was used for the project.

A detailed history was taken and physical examination was done and recorded after taking consent from parents/guardians of these patients.

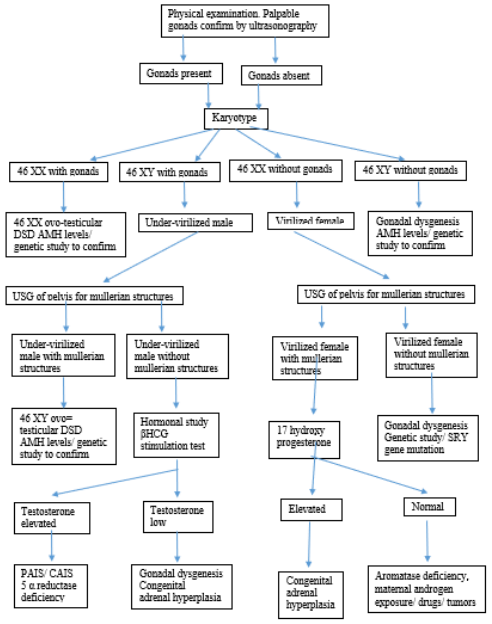

Figure 1: *Diagnostic algorithm for disorders of sexual development (DSD)*
AMH: Anti-Mullerian hormone, HCG: Human chorionic gonadotrophin, CAIS: complete androgen insensitivity syndrome, PAIS: partial androgen insensitivity syndrome

Chromosomal gender was assigned based on the karyotype. An ultrasonogram (USG) was done to identify gonads, Wolffian duct remnants, Mullerian remnants and other malformations. Hormonal and biochemical tests as mentioned in table 3 were performed based on the individual clinical profile. Magnetic Resonance Imaging (MRI) of the pelvis was done in one case to identify remnants of Mullerian duct as these were not visualized in the USG of the pelvis.

The clinical characteristics of the 7 patients are shown in Table 1. The ages ranged from 7 days of life to 5 years of age; 6 (85%) were products of a consanguineous marriage; 6 (85%) were perceived as males by the parents. After karyotyping, the chromosomal gender was male in 3 (43%) patients.

Patients were evaluated by routine investigation (Table 2) and then more specific hormonal studies, biopsy and genetic testing were done in selected patients (Table 3).

Table 1: ***Clinical characteristics of patients***

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Karyotype	46, XX	46, XX	46, XX	46, XY	46, XY	46, XY	46, XX
Age	3 months	5 years	4 months	3 years	6 months	5 months	7 days
Birth order	2nd	3rd	2nd	1st	1st	1st	2nd
Consanguinity	3rd degree	2nd degree	3rd degree	No	2nd degree	3rd degree	3rd degree
Parental perception of gender	Female	Male	Male	Male	Male	Male	Male
Physical characteristics	Enlarged clitoris (SPL 2cm) Single vaginal orifice Labia developed and fused	Short stature (Ht. 99cm ≤3Z) Ambiguous genitalia, small phallus (SPL 2.5 cm) Penoscrotal hypospadias with bifid, empty scrotum	Small phallus with coronal hypospadias	Bifid scrotum Penoscrotal hypospadias.	Bifid scrotum Small phallus (SPL 2cm) Penoscrotal hypospadias	Small phallus (SPL 2cm) Empty scrotum with no rugosity and no gonads. No vaginal opening	Enlarged phallus Perineal meatus Fused labia
Gonads	Not palpable	Rt. testis palpable in Rt. inguinal canal Lt. testis not palpable	Rt. testis palpable in scrotum Lt. scrotal sac empty and fused with the phallus (Figure 4)	Lt. testis palpable in scrotum Rt. testis palpable in inguinal canal	Both gonads palpable in inguinal canal	Not palpable	Not palpable
Other physical features	No hyperpigmentation, hypertension or dysmorphism	No hyperpigmentation, hypertension or dysmorphism	No hyperpigmentation, hypertension or dysmorphism	No hyperpigmentation, hypertension or dysmorphism	No hyperpigmentation, hypertension or dysmorphism	No hyperpigmentation, hypertension or dysmorphism	Hyperpigmented genitalia

SPL: stretched penile length

Table 2: ***Investigations of the patients***

Characteristic	Patient 1	Patient 2	Patient3	Patient 4	Patient 5	Patient 6	Patient 7
Electrolytes (mEq/L)	Sodium -147 Potassium-4.9	Sodium -141 Potassium - 4.2	Sodium - 137 Potassium -3.9	Sodium - 140 Potassium - 4.5	Sodium - 146 Potassium - 4.2	Sodium -140 Potassium - 3.5	Hyponatremia (Sodium -127) Hyperkalaemia (Potassium - 6.7) Salt wasting, shock and hypoglycaemia
Ultrasonography of pelvis and scrotum	Both ovaries and adnexa visible	Bilateral empty scrotal pouches, Rt. testes visible in Rt. inguinal canal. Lt testes and Mullerian remnants not visualised	Hernial sac visualized on Rt. side with Rt. gonad, likely testes. Uterus and vagina visualized with VVF, with an intraabdominal gonad on Lt. side, likely ovotestes.	Lt. testis seen in scrotal sac. Rt. testis seen in superficial inguinal ring. No mullerian structures seen	Both testes seen in inguinal canal. No Mullerian structures seen	Two hypoechoic oval lesions visualized in suprapubic areas, likely to be undescended testes. Mullerian remnants not visualized	Uterus visualised along with rudimentary ovaries. Testes not present.
Magnetic resonance imaging of pelvis		Oblong cystic structure in pelvis likely to be cystic remnant of Mullerian duct. Bilateral undescended testes seen in both inguinal canals					
Karyotype	46 XX	46 XX	46 XX	46 XY	46 XY	46 XY	46XX

VVF: vesico-vaginal fistula

Table 3: ***Hormonal, genetic and other studies of patients***

Characteristic	Patient 1		Patient 2	Patient 3	Patient 4		Patient 5		Patient 6		Patient 7
	Pre stimuln.	Post stimuln.			Pre stimuln.	Post stimuln.	Pre stimuln.	Post stimuln.	Pre stimuln.	Post stimuln.	
17-OHP (ng/ml) Normal: 0.25-1.1	1.05	4.42	0.36	0.15					4.495	5.42	54
ACTH (pg/ml) Normal: 0-46	16	.1250									
Cortisol (µg/dl) Normal: 3-21	5.1	29									
DHEA (ng/ml) Normal: 1.2-6	2.48	4.81									
Androstenedione (ng/ml) Normal:0.05-0.35	<0.3	<0.3							0.3	0.66	
Progesterone (ng/ml) Normal: 0-0.61	<0.1	0.8									
DHT (pg/ml) Normal: 250-990					34.98	42.68	349.8	1066.8	118.18	189.94	
Testosterone(ng/ml) Normal: 2-25				0.41	226.16	936.19	3.6	279.26	0.22	2.3	
LH (IU/L) Normal: 0.5-10									0.4	<0.1	
FSH (IU/L) Normal: 1.3-11.5									1.96	0.59	
DHEAS µg/dl Normal: 24.4-209.7			3.2								
Oestradiol pg/ml Normal: 11-44			<5	14							
AMH (ng/ml) Normal: 2-6.8			21.24	26.23							
Genetic study/ Other study	NGS showed homozygous nonsense variation in exon 10 of CYP19A1 gene, a pathological variant of aromatase deficiency, resulting in defective conversion of dehydroepiandrosterone sulphate to oestrogen by fetus, and its subsequent conversion to testosterone peripherally resulting in virilisation of female fetus		Biopsy- Both gonads showed cords of seminiferous tubules in an ovarian stroma suggestive of ovotestes	Biopsy- Both gonads showed cords of seminiferous tubules in an ovarian stroma suggestive of ovotestes	Diagnosis was confirmed by characteristic physical examination and presentation of child, supported by karyotype, absence of mullerian structures and hormonal study result. A high level of testosterone both pre and post stimulation and a very low level of dihydrotestosterone both pre and post stimulation.		Diagnosis was confirmed by characteristic physical examination and presentation of child, supported by karyotype, absence of mullerian structures and hormonal study result. A normal level of testosterone and dihydrotestosterone along with a significant rise in both testosterone and dihydrotestosterone levels post stimulation in a 46 XY child with under virilisation is suggestive of AIS.		Diagnosis was confirmed by characteristic physical examination and presentation of child, supported by karyotype, absence of mullerian structures and hormonal study result. Very low levels of testosterone and dihydrotestosterone along with an insignificant rise in both testosterone and dihydrotestosterone levels post stimulation in a 46 XY child with under virilisation is suggestive of testicular dysgenesis		NGS showed a non-synonymous variant in NM_0005000.7: c.955C>T (p. Gln319Ter)CYP 21A2 gene in homozygous state. The gene CYP21A2 encodes 21 hydroxylase essential for steroidogenesis.
Final diagnosis	Aromatase deficiency		46 XX Ovo testicular DSD	46 XX Ovo testicular DSD	5α Reductase deficiency		Androgen insensitivity syndrome		Testicular dysgenesis		Congenital adrenal hyperplasia 21 hydroxylase deficiency

17-OHP: 17-hydroxy progesterone, ACTH: adrenocorticotrophic hormone, DHEA: dehydroepiandrosterone, DHT: dihydrotestosterone, LH: Luteinising hormone, FSH: Follicle stimulating hormone, DHEAS: dehydroepiandrosterone sulphate, AMH: anti-Mullerian hormone, NGS: next generation sequencing, AIS: androgen insensitivity syndrome, DSD: disorders of sexual differentiation

Discussion

DSDs are a diverse group of disorders. The disorders mentioned in our case series are very rare like aromatase deficiency (only a few cases reported worldwide)[7], 46XX ovo-testicular DSD (1 per 20,000)[8], androgen insensitivity syndrome (2 to 5 per 100,000)[9] and 5 alpha reductase deficiency (incidence unknown)[9].

Features suggestive of DSD include cases with overt genital ambiguity, apparent male genitalia with bilateral cryptorchidism, hypospadias with unilateral cryptorchidism, severe cases of hypospadias like penoscrotal, perineal or scrotal, apparent female genitalia with enlarged phallus or inguinal hernia or palpable testes and cases with anatomical or karyotypic discordance[6].

A thorough clinical history and detailed clinical examination is the cornerstone of diagnosing DSD. Details should be taken about consanguinity and family history of similar disorders in other members. Clinical examination traditionally starts with the presence or absence of palpable gonads along with measurement of length of phallus, and noting the presence of hyperpigmentation and dysmorphism. This should be followed by initial investigations like fluorescence *in situ* hybridization (FISH) /polymerase chain reaction (PCR) for early detection of XY chromosomes followed by complete karyotype to look for mosaicism. It should also include assessment of internal organs by USG and if required MRI should be done to look for intra-abdominal organs. Further evaluation should be directed as given in the simplified algorithm (Figure 1)

Genetic testing like microarray is used to look for deletions in infants with syndromic DSD[5]. Newer techniques like whole exome sequencing and next generation sequencing help in specific diagnoses of previously undiagnosable cases.

Conclusions

There were 2 cases of 46, XX ovo-testicular DSD and 1 case each of aromatase deficiency, congenital adrenal hyperplasia, 5 alpha reductase deficiency, testicular dysgenesis and androgen insensitivity syndrome.

Acknowledgments

The authors thank the patients and their families for their participation.

References

1. Warne GL, Raza J. Disorders of sex development (DSDs), their presentation and management in different cultures. *Reviews in Endocrine and Metabolic Disorders* 2008; **9**(3): 227-36. https://doi.org/10.1007/s11154-008-9084-2 PMid: 18633712
2. Kolesinska Z, Ahmed SF, Niedziela M, Bryce J, Molinska-Glura M, Rodie M *et al.* Changes over time in sex assignment for disorders of sex development. *Pediatrics* 2014; **134**(3): e710-e715. https://doi.org/10.1542/peds.2014-1088 PMid: 25092939
3. Arboleda VA, Sandberg DE, Vilain E. DSDs: genetics, underlying pathologies and psychosexual differentiation. *Nature Reviews*

Endocrinology 2014; **10**: 603-15. https://doi.org/10.1038/nrendo.2014.130 PMid: 25091731 PMCid: PMC4441533

4. 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(2): e488-500. doi: 10.1542/peds.2006-0738. PMID: 16882788. https://doi.org/10.1542/peds.2006-0738 PMid: 16882788
5. Wherrett DK. Approach to the Infant with a suspected disorder of sex development. *Pediatric Clinics of North America* 2015; **62**(4): 983-99. https://doi.org/10.1016/j.pcl.2015.04.011 PMid: 26210628
6. Walia R, Singla M, Vaiphei K, Kumar S, Bhansali A. Disorders of sex development: a study of 194 cases. *Endocrine Connections* 2018; 7(2): 364-71. https://doi.org/10.1530/EC-18-0022 PMid: 29386228 PMCid: PMC5825923
7. Reserved IU-A. Orphanet: Aromatase deficiency. [cited 2022 Sep 21]. Available from: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Lng=GB&Expert=91
8. Ovotesticular disorder of sex development - NORD (national organization for rare disorders) 2015 [cited 2022 Sep 21]. Available from: https://rarediseases.org/rarediseases/ovotesticular-disorder-of-sex-development/
9. Zhu YS, Imperato-McGinley J. Androgen insensitivity syndrome. In: Encyclopaedia of Endocrine Diseases. Elsevier; 2004. p. 214–20. https://doi.org/10.1016/B0-12-475570-4/00098-6
10. 5-alpha reductase deficiency. Medlineplus.gov. [cited 2022 Sep 21]. Available from: https://medlineplus.gov/genetics/condition/5-alpha-reductase-deficiency/