Gender outcome in children with disorders of sex development: A cryptic misfortune

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Received – 21 November 2016 Initial Review – 31 December 2016 Published Online – 25 January 2017

ABSTRACT

Background: Disorders of sex development (DSD) are an important cause of management dilemma for a clinician, especially due to the difficulty in assigning a suitable sex to them. Not many studies are reported regarding their gender outcome. **Objective:** To find out the gender outcome of children with DSD. **Materials and Methods:** A hospital-based descriptive study was conducted in children with DSD from birth till 12 years of age who were attending pediatric outpatient department and ward and endocrinology clinic of a tertiary care teaching hospital of South India for 1 year. Children with DSD who were registered in endocrinology clinic over the preceding 10 years were called for review. Those came for review were also included. They were analyzed for their assigned gender at birth, etiological diagnosis, current gender role and phenotype, treatment and follow-up patterns. Reinvestigations were done in needed cases. **Results:** A total of 38 cases were analyzed in the study. Work up could be completed in 92.1% of children. 60.5% cases were diagnosed in infancy, and 18.42% (n=7) of cases were identified above 5 years. Among those who were not assigned any sex at birth, 50% became phenotypic male and 50% became phenotypic female. 25% of the patients, who were assigned male sex at birth, changed to female sex. 100% of 46 XX DSD are being reared as females but only 44.4% of 46 XY patient are being reared as males. **Conclusion:** Sex assignment in DSD, especially 46 XY DSD, is a great challenge. Sex assignment must be based on a definitive etiological diagnosis, its natural course, gender role, gender identity, external genital structure and reproductive outcome and with proper counseling of the parents. Strict follow-up is inevitable.

Key words: *Children, Disorders of sex development, Etiological diagnosis of disorders of sex development, Gender outcome*

Disorders of sex development (DSD) may be the most devastating condition after perinatal mortality, to face by any parent of a newborn, not only with the uncertainty involved in determining the sex of the child, but also with the social stigma attached to it [1-3]. DSD or the transgender as it is called now may be identified in later childhood or after marriage or even after evaluating for infertility in a couple. Although legally the term transgender is going to be in records, their psychosocial and sexual problems cannot be solved with our existing social conditions. No single protocol is available for treatment and in the most cases even workup may not be fully completed. When the gender of an intersex child was wrongly assigned at birth, subsequent gender re-assignment creates some of the most emotional and contentious ethical dilemmas encountered in any area of medical practice. Due to the social stigma attached to the condition, many won't turn up for follow-up. We cannot construct male genitalia. Hence, to avoid a change in sex later, it is necessary to predict the pubertal change, sexual outcome, gender identity and gender role in any case of DSD so that they can be reared in the most suitable sex for them.

There are no solid long-term outcome studies on patients managed with intersex disorders in current literature. In this context, we tried to find out the gender outcome of children with DSD in our hospital, for emphasizing the social emergency of the situation.

MATERIALS AND METHODS

The research protocol of the study was submitted to the Research and Human Ethical Committee of the institution. The study was started after getting the clearance from both the committees. Children from birth up to 12 years of age, who fulfilled the criteria for DSD according to the standard literature [4,5] and who came to our pediatric outpatient (OP) clinic, pediatric wards and endocrinology clinic during 1-year period (1st January 2010 to 31st December 2010) were enrolled. All the cases of DSD in the endocrinology OP registry over 10-year period (1st January 2000-31st December 2009) were called for both by letter and telephone. All consecutive cases who responded to the call were enrolled in the study after getting an informed consent. Hence, 28 old cases were analyzed and evaluated. 10 fresh cases were recruited over the study period. Thus, a total of 38 patients were enrolled in the study.

A pro forma was prepared with personal data including age at first presentation, current age, assigned gender at birth, sex

of rearing, gender identity, consanguinity, antenatal history and drugs, family history, initial presenting complaints, general examination, hyperpigmentation, state of hydration, auxology, blood pressure, description of genitalia at presentation (from old records also), initial investigations - laboratory/karyotyping/imaging/laparoscopy/biopsy), initial diagnosis and treatment received (medical/surgical), current appearance of external genitalia (Prader staging), reinvestigations done if any, revised diagnosis if any, treatment planned, follow-up pattern (regular/irregular) and reason for irregular follow-up.

The genital ambiguity inclusive of the symmetry of the labioscrotal folds, labial fusion, phallic length, morphology of the phallus, number of urogenital openings, palpable gonads, and the site of gonad, position of urethral opening, chordee and anogenital ratio were recorded to describe the stage of virilization. A newborn was considered to have DSD when the baby satisfied the criteria as shown in Table 1 [4,5].

Laboratory investigations included serum electrolytes, random blood sugar, renal function and electrolytes, hormonal studies like basal concentration of 17-hydroxyprogesterone (17-OHP), testosterone, dihydrotestosterone (DHT), dehydroepiandrosterone, follicle stimulating hormone, and luteinising hormone according to the clinical diagnosis. In doubtful cases, adrenocorticotropic hormone (ACTH) stimulation test was done with an intravenous bolus of 0.125 mg of ACTH to observe the rise in 17-OHP (one of our cases needed it). However, 11 deoxycortisol or deoxycorticosterone was not available in our settings to type the congenital adrenal hyperplasia (CAH). In some cases, human chorionic gonadotropin (hCG) stimulation test was done after 3 months by giving 1000 units hCG IM on alternate days for 3 doses to study the levels of testosterone and DHT.

Table 1: Clinical definitions and criteria [4,5]

DSD: Atypical development of genital, gonadal and phenotypic sex with respect to chromosomal sex
Ambiguous genitalia: When there is difficulty in assigning sex to an individual from the appearance of external genitalia or when phenotypic sex is neither completely male nor female
DSD in the newborn period: (1) A phallus and bilaterally non palpable testis, (2) unilateral cryptorchidism and hypospadias, (3) penoscrotal or perineoscrotal hypospadias with or without microphallus even if the testis are descended, (4) discordance of external genitalia compared with prenatal karyotype, (5) apparently female appearance with enlarged clitoris or inguinal hernia, (6) overt genital ambiguity such as cloacal extrophy, (7) asymmetry of labioscrotal folds with or without cryptorchidism
Prader staging: Complete female (Type 5), partial female (Type 4), ambiguous (Type 3), predominantly male with micropenis (Type 2), and completely male phenotype without apparent undervirilization (Type 1)
Gender role: It is a set of societal norms dictating what types of behaviours are generally considered acceptable, appropriate, or desirable for a person based on their actual or perceived sex
Gender identity: Personal conception of oneself as male or female (or both or neither)

DSD: Disorders of sex development

Pelvic and abdominal ultrasonography helped to detect the presence of gonads and mullerian structures. Other investigations included genitogram, laparoscopy, and gonadal biopsy. For older cases, the previous investigations were analyzed, re-examination was done and needed investigations were conducted to arrive at a definitive diagnosis. Polymerase chain reaction for SRY or mutation analysis for enzyme defects was not available in our settings. Karyotyping was done for all patients on peripheral blood lymphocytes. From the clinical examination and the investigations, we arrived at the etiological diagnosis of DSD following the standard protocols [6].

RESULTS

In the study group, the age at diagnosis ranged from birth till 12 years. 50% (n=19) of the cases were identified in the newborn period itself. An additional 10.5% (n=4) cases were identified before 1 year; hence, a total of 60.5% cases were diagnosed in infancy. 21.05% (n=8) and 18.42% (n=7) of cases were identified between 1 and 5 years and after 5 years of age, respectively. The cases presented after 5 years included 3 cases of simple virilizing type of CAH and 2 cases of complete androgen insensitivity syndrome (CAIS) and 2 cases of partial androgen insensitivity syndrome (PAIS). According to the clinical, laboratory and imaging studies, the 38 cases were classified as per the latest recommendations as shown in Table 2 [6]. The majority of the cases were CAH followed by CAIS.

At birth, 22 (57.89%) children were assigned as females, 8 (21.05%) were assigned as male and 8 (21.05%) cases were not assigned any sex. Presently, 28 (73.7%) cases are being reared

Table 2: Etiological classification [6]

Type	Frequency (n=38)
46, XX DSD	17 (44.7)
CAH-salt losing	8 (21.1)
CAH-simple virilizing	8 (21.1)
Genital malformation	1 (2.6)
46, XY DSD	17 (44.7)
CAIS	6 (15.79)
PAIS	3 (7.9)
5 alfa reductase deficiency	3 (7.9)
Pure gonadal dysgenesis	1 (2.6)
PMDS	1 (2.6)
Incomplete work up	3 (7.9)
Sex chromosome DSD	2 (5.26)
47 XXY	1 (2.63)
49XXXXY (50)/48XXXY (30)/46XY (20)	1 (2.63)
Ovotesticular DSD	2 (5.26)
46, XY	1 (2.63)
46XX/46XY	1 (2.63)
46 XX testicular DSD	0 (0)

CAH: Congenital adrenal hyperplasia, CAIS: Complete androgen insensitivity syndrome, PAIS: Partial androgen insensitivity syndrome, PMDS: Persistent mullerian duct syndrome, DSD: Disorders of sex development, CAH: Congenital adrenal hyperplasia

as females and only 10 cases (26.3%) as males. Among those who were assigned as females, 100% continued to be so. Among those who were assigned as males, 25% (n=2) changed to female sex. Among those who were not assigned any sex at birth, 50% became phenotypic male and 50% became phenotypic female. Hence, there was a significant proportion of change in sex after initial assignment. Table 3 shows the trend of change in sex.

Assigned sex at birth and sex of rearing according to the various etiological diagnoses and according to the chromosomal sex are given in Tables 4 and 5, respectively.

DISCUSSION

For a proper gender identity and a gender role in the community, it is better to have no change in sex after 2 years because in humans, environment also is a powerful mediator of gender identity and behavior [6-8]. In our study, the cases presented after 5 years of age included androgen insensitivity syndromes (Fig. 1) and simple virilizing type of CAH. The later ones were XX only, and they can be continued as females if proper treatment is planned. However, the former ones created the worst mental stress, initially for the parents and later to the kids as well, as they knew that they are genetically males.

Change of gender from that at birth is an important matter for discussion. Although all who were assigned female sex at birth continued to be so, many cases of CAH who were detected earlier and with no regular follow-up, came with abnormal looking genitals (Fig. 2). Many of them have partial male gender identity as well. Among those who were assigned male sex, two cases of CAH changed to female later. Among those who were not assigned any sex at birth, 50% each became males and females (Tables 3 and 4). Hence, in doubtful cases, do not label these children any sex till the evaluation is completed.

Knowing the etiology can predict the gender outcome. Among 46, XX CAH, at birth 81.25% of babies was assigned female sex, 12.5% were assigned male sex and 6.25% were undetermined. However, all are now being reared as females. In a study by Al-Mulhaim et al., all the newborns with 46 XX CAH were raised as females and those with delayed presentation of CAH were reared as male [9]. That signifies the importance of identifying XX CAH earlier because severe variety may get misinterpreted as males at birth and cannot be reared as females if they are presenting very late because they will be having male gender identity and gender role due to the androgenic influence on brain [10,11]. However, a correctly managed XX CAH can have a normal life with normal reproductive functions. Those who are being reared as males will be impotent, will not be having a complete male phenotype and finally will be identified in community as transgender. 46 XX with local genital malformation can also be improved by genital reconstruction. Hence, this study emphasizes that all XX DSDs should be reared as females only [12,13].

Due to the varied etiology, assigning sex to a 46 XY DSD needs proper thinking and evaluation. No single protocol is available. In our study, those XY children who were raised as females included CAIS, PAIS, and pure gonadal dysgenesis. In a study by Gollu et al., those XY raised as females included CAIS, PAIS and 5-α reductase deficiency [14]. All cases of CAIS were assigned female sex at birth and now also being reared as females. They have undergone vaginoplasty and testes were removed. Among PAIS, 66.6% were assigned female sex and 33.3% had an undetermined sex at birth but all are now being reared as females. All of them underwent genital reconstruction procedures but are not under correct follow-up. About 66.6% of children with 5-α reductase deficiency were assigned undetermined sex, and 33.3% were assigned male sex at birth. All are now being reared as males.

Only one case of PMDS (Fig. 3) was there who was assigned male sex at birth and was also raised as male. Child had bilateral cryptorchidism which was ignored initially and at 5½ years

Table 3: Relationship between assigned sex at birth and sex of rearing

Assigned gender at birth	Gender of rearing (%)	
	Male	Female
Male (n=8)	75 (n=6)	25 (n=2)
Female (n=22)	0 (n=0)	100 (n=22)
Undetermined sex (n=8)	50 (n=4)	50 (n=4)

Table 4: Assigned sex at birth and sex of rearing of various etiological types of DSD

Aetiological types of DSD	Assigned sex at birth (%)			Gender of rearing (%)	
	Female	Male	Undetermined	Female	Male
CAH (n=16)	81.25 (n=13)	12.5 (n=2)	6.25 (n=1)	100	0
CAIS (n=6)	6 (100%)	0	0	100	0
5-α reductase deficiency (n=3)	0	33.33 (n=1)	66.66 (n=2)	0	100
Sex chromosome DSD (n=2)	0	50 (n=1)	50 (n=1)	0	100
PAIS (3)	66.66 (n=2)	0	1 (100)	100	0
PGD	1 (100)	0	0	100	
PMDS	0	1 (100)	0	0	100
Ovotesticular DSD	0	1 (50)	1 (50)	50	50
Workup incomplete XY	0	66.66 (n=2)	33.33 (n=1)	0	100
46, XX genital malformation	0	0	1 (100)	100	0

CAH: Congenital adrenal hyperplasia, CAIS: Complete androgen insensitivity syndrome, PAIS: Partial androgen insensitivity syndrome, PMDS: Persistent mullerian duct syndrome, DSD: Disorders of sex development, CAH: Congenital adrenal hyperplasia

Table 5: Chromosomal sex and sex of rearing

Chromosomal sex	Sex of rearing (%)	
	Male	Female
46 XX (n=17)	0 (n=0)	100 (n=17)
46 XY (n=18)	44.4 (n=8)	55.6 (n=10)
Abnormal karyotypes (n=3)	66.7 (n=2)	33.3 (n=1)

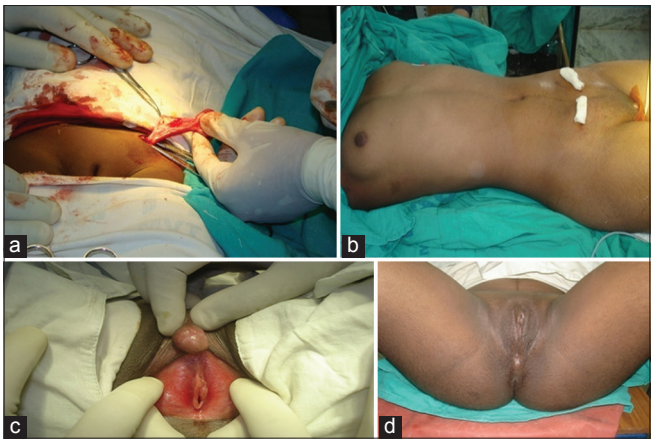

Figure 1: A case of partial androgen insensitivity syndrome, 12-year-old sibling of a case of complete androgen insensitivity syndrome, when brought up for inguinal swelling and clitoromegaly (a) Inguinal mass was proven to be testes, (b) normal breast development, (c) normal vaginal orifice (but was a blind ending loop) and penile like phallus, (d) review after clitororeduction

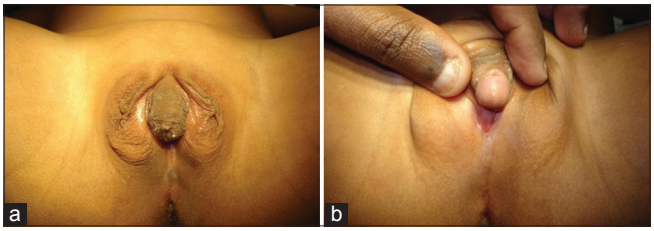

Figure 2: A case of 46, XX congenital adrenal hyperplasia salt wasting type with ambiguous genitalia. (a) Clitoromegaly and the rugouslabioscrotal folds, (b) a well-developed vaginal orifice

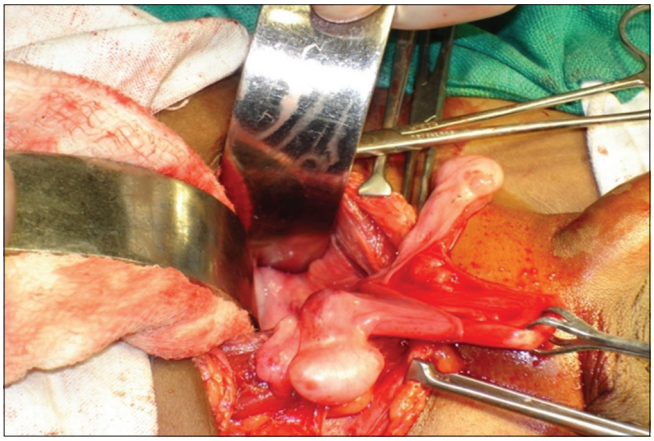

Figure 3: A case of persistent mullerian duct syndrome who presented with bilateral cryptorchidism. Ultrasound showed mullerian structures. Inguinal exploration showed fallopian tubes along with testes

inguinal exploration revealed testes and mullerian structures (fallopian tubes). Same was removed and now being reared as male. One case of XY pure gonadal dysgenesis was assigned female from birth. The child had ambiguity of genitals at birth and was not evaluated and presented with clitoromegaly and right inguinal swelling at the age of 3 months. Karyotype was 46, XY and ultrasound and laparoscopy revealed rudimentary uterus with bilateral fallopian tubes. Testicular biopsy revealed bilateral hyalinized testes. Gonadectomy and feminizing genitoplasty were done and now the child is being reared as female. The cases of XY (n=3) with incomplete workup were raised as males.

Combining all, only 44.4% of 46, XY patients are being reared as males. So before assigning a sex to any XY DSD, we should try to reach a correct diagnosis, should know the natural course of the etiological type and its pubertal change. A rough guideline is as follows [7,12,15].

- All cases of CAIS should be reared as females as their external genitals are female looking and have female phenotype. Although they won't be a fertile female, they can have a sexual life after proper vaginoplasty.
- In PAIS or gonadal dysgenesis - If phallic length is more than 1.9 cm and administration of testosterone enanthate/ cypionate 25 mg IM 3-4 injections at 3-4 weeks intervals increases penile length to 2.5 cm, rear as male. In a term infant, if phallic size is <1.5 cm long and 0.7 cm wide, it is preferred to rear as female [13].
- Those XY DSD who are being reared as females should be provided with prepubertal and pubertal estrogen therapy for a better looking female phenotype. They may need to be repeated genitoplasty and vaginoplasty and should be counseled regarding fertility issues.
- Testicular biosynthetic defects like 17 ketosteroid reductase deficiency and 5-α reductase deficiency, even if reared as females, will adopt a male gender role at puberty due to the coincidental increase in Testosterone and DHT to typical male levels at puberty and presumably due to the direct action of testosterone on the phallus [16-18]. Therefore, they should be reared as males only, and therefore, differentiating PAIS from 5-α reductase deficiency at the earliest is very important [15].

In sex chromosome DSD, we had 2 abnormal karyotypes [19]. 47 XXY and its variant form (49 XXXXY (50)/48 XXXY (30)/46 XY (20)). Both are being reared as males. 49 XXXXY (50)/48 XXXY (30)/46 XY (20) is considered to be a mosaic variant of Klinefelter syndrome. It is very rare to find such a mosaicism. Mosaic forms are due to mitotic nondisjunction after fertilization of the zygote. These forms can arise from a 46XY zygote or a 47 XXY zygote.

In ovotesticular DSD, 50% (n=1) each were raised as males and females. Ovotesticular DSD is the rarest variant making only up to 10% of intersex disorders. About 70% have a 46, XX karyotype, fewer than 10% have 46, XY and about 20% have 46, XX/46, XY [20]. In our study, the karyotypes were 46 XY and 46 XX/46 XY. The 46 XY ovotesticular DSD is a rare variety, and our baby presented with ambiguous genitalia at birth. On

examination, had microphallus, the gonads were at the root of scrotum and biopsy revealed bilateral ovotestes. Although being reared as male, the genitals were still ambiguous. The second one was 46 XX/46 XY chimerism of which, only few cases are reported in the literature. 46 XX/46 XY chimer is an individual in whom some cells have the male chromosome complement, and some have female. When more than one set of cell line with different sets of chromosomes make up the body, it is called chimerism. It occurs when two non-identical zygotes combine at a very early stage of development to form a single fetus. It is different from mosaicism where the individual originates from a single fetus [21]. In our study, this case was a phenotypic girl who presented at the age of 4 years with left inguinal swelling and microphallus and hypospadias. Gonadal biopsy revealed ovotestes with fibromuscular changes on the right side and testes on the left side. She had undergone genital reconstruction. In ovotesticular DSD, if highly virilized, rear as male if there are good testicular function and no uterus. Otherwise remove the inappropriate gonad and rear as female.

So in a nutshell, all DSDs should be managed after detailed discussion with parents and also with children when appropriate, based on the karyotype, endocrine function, genital anatomy, expected pubertal change and fertility issues, to assign a sex for rearing. The family and the doctor must accept that any treatment strategy may fall short of a completely normal child. A proper collaboration between medical, surgical, radiology, laboratory, social and psychology wings provides the best possible outcome. Proper counseling of the parents during initial presentation is the only way to remove the misconcepts and also to empower them with proper knowledge so that most of them will turn up for regular treatment.

There were few limitations for our study. The new cases detected during newborn period could not be followed up beyond 1 year and fallacies for hormonal studies can occur during earlier months. Furthermore, many of our children have not reached pubertal age group, which is a crucial time for determining gender outcome.

CONCLUSION

Although major proportion of DSDs was identified in newborn period, 18% were identified after 5 years only, and they were CAH and androgen insensitivity syndromes. It is difficult to complete the workup in many cases of DSDs in our setting. Change of sex from those assigned at birth and change in gender role and identity were more for XY DSD. Only 44.4% of them are being reared as males. All those who are identified as females at birth (both XX and XY) and all XX DSDs are being reared as females only.

ACKNOWLEDGMENT

Dr. V H Sankar for scientific support and Dr. C S Aravind for photography.

REFERENCES

1. Aaronson IA. The investigation and management of the infant with ambiguous genitalia: A surgeon's perspective. Curr Probl Pediatr. 2001;31(6):168-94.
2. Nthumba PM, Carter LL, Poenaru D. Ambiguous genitalia in rural Africa and the complexities of management: Which way forward? East Cent Afr J Surg. 2008;13(1):51-8.
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(2):151-66.
4. Rosolowsky ET, spack NP. Disorders of sex development. In: John PC, Eric CE, Ann RS, editors. Manual of Neonatal Care. Vol. 30. New Delhi: Wolters Kluwer Ltd.; 2009. p. 574-85.
5. Coran AG, Polley TZ Jr. Surgical management of ambiguous genitalia in the infant and child. J Pediatr Surg. 1991;26(7):812-20.
6. Hughes IA, Houk C, Ahmed SF, Lee PA; Lawson Wilkins Pediatric Endocrine Society/European Society for Paediatric Endocrinology Consensus Group. Consensus statement on management of intersex disorders. J Pediatr Urol. 2006;2(3):148-62.
7. Mouriquand PD. Possible determinants of sexual identity: How to make the least bad choice in children with ambiguous genitalia. BJU Int. 2004;93 Suppl 3:1-2.
8. Houk CP, Lee PA. Intersexed states: Diagnosis and management. Endocrinol Metab Clin North Am. 2005;34(3):791-810, xi.
9. Al-Mulhim AN, Kamal HM. Ambiguous genitalia in neonates: A 4-year prospective study in a localized area. East Mediterr Health J. 2010;16(2):214-7.
10. Nordenström A, Servin A, Bohlin G, Larsson A, Wedell A. Sex-typed toy play behavior correlates with the degree of prenatal androgen exposure assessed by CYP21 genotype in girls with congenital adrenal hyperplasia. J Clin Endocrinol Metab. 2002;87(11):5119-24.
11. Berenbaum SA, Bailey JM. Effects on gender identity of prenatal androgens and genital appearance: Evidence from girls with congenital adrenal hyperplasia. J Clin Endocrinol Metab. 2003;88(3):1102-6.
12. Clayton PE, Miller WL, Oberfield SE, Ritzén EM, Sippell WG, Speiser PW, et al. Consensus statement on 21-hydroxylase deficiency from the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society. Horm Res. 2002;58(4):188-95.
13. Rajendran R, Hariharan S. Profile of intersex children in south India. Indian Pediatr. 1995;32(6):666-71.
14. Göllü G, Yildiz RV, Bingol-Kologlu M, Yagmurlu A, Senyücel MF, Aktug T, et al. Ambiguous genitalia: An overview of 17 years' experience. J Pediatr Surg. 2007;42(5):840-4.
15. Thomas DF. Gender assignment: Background and current controversies. BJU Int. 2004;93 Suppl 3:47-50.
16. Patel ZM, Ambani LM, Dalal SJ, Gokarn VV, Desai MP, Rao CS, et al. Ambiguous genitalia. Indian Pediatr. 1983;20(4):279-84.
17. Reiner WG, Gearhart JP. Discordant sexual identity in some genetic males with cloacal exstrophy assigned to female sex at birth. N Engl J Med. 2004;350(4):333-41.
18. Imperato-McGinley J, Peterson RE, Gautier T, Sturla E. Androgens and the evolution of male-gender identity among male pseudohermaphrodites with 5alpha-reductase deficiency. N Engl J Med. 1979;300(22):1233-7.
19. Lambert SM, Vilain EJ, Kolon TF. A practical approach to ambiguous genitalia in the newborn period. Urol Clin North Am. 2010;37(2):195-205.
20. White PC. Disorders of the adrenal glands. In: Behrman RE, Kliegman RM, Jenson MD, editors. Nelson Textbook of Pediatrics. 18th ed. Philadelphia, PA: WB Saunders Company; 2008. p. 2360-8.
21. Yordam N, Alikasifoglu A, Kandemir N, Caglar M, Balci S. True hermaphroditism: Clinical features, genetic variants and gonadal histology. J Pediatr Endocrinol Metab. 2001;14(4):421-7.

Funding: None; Conflict of Interest: None Stated.

How to cite this article: Rahul A, Saraswathyamma S. Gender outcome in children with disorders of sex development: A cryptic misfortune. Indian J Child Health. 2017; 4(2):114-118.