Original Article

"Spectrum of 46 XY Disorders of Sex Development": A Hospital-based Cross-sectional Study

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Abstract

Background: Disorders of sex development (DSD) are a wide range of relatively rare conditions having diverse pathophysiology. Identification of an underlying cause can help in treating any coexisting hormone deficiencies and can help with anticipating any other immediate or long-term health concerns. **Objective:** To study the clinical and biochemical profile of patients with 46 XY DSD along with androgen receptor (AR) gene mutation status in selected group of patients. **Methods:** A cross-sectional study was conducted after enrolling the eligible DSD patients. Thorough elicitation of history and detailed clinical examination was done. Assays for luteinizing hormone, follicle-stimulating hormone, testosterone, dihydrotestosterone, androstenedione, AMH & Inhibin B (where indicated), and human chorionic gonadotropin stimulation were done as per protocol. **Results:** In total, 48 patients were included in the study. Ambiguous genitalia (58.3%) followed by hypospadias (33.3%) were common presentation. Androgen biosynthetic defect were the most commonly encountered diagnosis followed by androgen insensitivity syndrome (AIS). Swyer syndrome was diagnosed in 4.2% of cases; partial gonadal dysgenesis, ovotesticular DSD, and vanishing testis syndrome contributed to 2% of cases each. Eight cases (16.7%) who presented with isolated proximal and midshaft hypospadias for whom no diagnosis was found were categorized in the "etiology unclear" group. AR gene mutation analysis designed against specific exons did not yield any results. **Conclusion:** 46 XY DSD is a heterogeneous group of patients with a varying age of presentation and a diverse clinical profile. Most patients are reared as males and maintained the same gender identity except in isolated cases. Diagnosis of AIS remains a clinical challenge as a definite hormonal criterion does not exist and genetic mutations in AR gene may be negative. Flanking region sequencing, whole genome sequencing, and promoter region sequencing may reveal pathogenic variants. Variations in other genes regulating AR pathway may also be candidates to be studied.

Keywords: Ambiguous genitalia, AR gene mutation, disorders of sex development, HCG stimulation test

Introduction

Disorders of sex development (DSD) are a wide range of relatively rare conditions having diverse pathophysiology. It is defined as "*congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical*".[1] The underlying etiology remains unclear in over half of these cases who have a 46 XY karyotype and are raised as males. 46 XY DSD is characterized by reduced androgenization, and the causes include complete or partial gonadal dysgenesis (CGD/PGD) or a defect in androgen synthesis or action. In 46 XY girls with a clinical diagnosis of androgen insensitivity syndrome (AIS), over 80% may have a mutation in the androgen receptor (AR) gene[2] in contrast to 20–30% in 46 XY boys. Thus, the commonest broad group of affected infants is boys with 46 XY DSD, and in this large group, around 60–70% do not have a confirmed genetic basis. Identification of an underlying cause can help with treating any coexisting hormone deficiencies and can help with anticipating any other immediate or long-term health concerns. There is some evidence that the long-term developmental outcome may

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be dependent on the underlying genetic diagnosis in 46 XY patients.[3,4]

Assam is a state of multiple ethnic and linguistic populations. There is a general paucity of information on DSD patients from this part of the country, particularly XY DSD. We present the clinical and biochemical profile of patients with 46 XY DSD presenting to the Endocrine clinic of a tertiary care center.

Methods

The present study was a cross-sectional study, conducted in the Department of Endocrinology of Gauhati Medical College, Guwahati, Assam, India, from January 2017 to June 2018. The study was approved by Institutional Ethical Committee. Consent was obtained from the parents of patients in case of children and patients themselves in case of adults after full explanation of the purpose and nature of all procedures used. Patients presenting with overt genital ambiguity, apparent male genitalia with nonpalpable testis, proximal or midshaft hypospadias, apparent female genitalia with clitoromegaly or female phenotype with primary amenorrhoea, and patients with inguinal/labial mass with a 46 XY karyotype were included. Thorough elicitation of history and detailed clinical examination including the external masculinization score (EMS) was done in all the patients. Blood samples were taken in fasting state for luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (T), dihydrotestosterone (DHT), androstenedione (A), and AMH & Inhibin B (where indicated). The serum samples were stored at -20°C till the final analysis. Human chorionic gonadotropin (hCG) stimulation was done as a routine protocol by administering hCG intramuscularly for 3 days (500 IU for infants, 1000 IU for children 1–10 yrs of age, and 1500 IU for >10 yrs) and sample for stimulated T, A, and DHT was collected 24 h after the last dose. A rise of plasma testosterone to above the upper limit of normal prepubertal range or rise by more than twice the baseline values was taken as a normal response.[5] Poststimulation T/A ratio of less than 0.8 was taken as diagnostic of 17β HSD deficiency[6] and poststimulation T/DHT ratio of more than 10 was taken as diagnostic of 5α reductase deficiency.[7] The diagnosis of androgen insensitivity was based on the presence of high-basal LH and T and/or two to three times rise of testosterone levels following hCG injection[8,9] and high-basal AMH. Gender identity, role, and behavior were assessed with the help of a clinical psychologist. The electrochemiluminescence immunoassay was used on Cobas E 411 immunoassay analyzers using Elecsys reagents for LH, FSH, and T. DHT and androstenedione were also measured by enzyme immunoassay using bioscience and Siemens Elisa kits. Intra- and interassay coefficient of variation was <5% with reference ranges for males of 0.16–1.69 mIU/ml (1 to <8 yrs), 0.21–2.80 mIU/ml (8 to <11 yrs), and 1.5–12.4 mIU/ml (adults) for FSH and <0.01–0.16 mIU/ml (1 yr to <8 yrs), <0.01–1.59 mIU/ml (8 to <11 yrs), and 1.7–8 mIU/ml (adults) for LH. For total, testosterone ranges were <0.02–0.11 ng/ml (1 to <7 yrs), <0.02–0.21 ng/ml (7 to <11 yrs), and 2.8–8 ng/ml for adults. For DHT and androstenedione, both intraassay and interassay CV were below 5% with ranges that vary as per age and tanner's stage. Serum AMH was done in patients with suspected AIS, isolated hypospadias, and anorchia. The electrochemiluminescence immunoassay "ECLIA" was used in cobas e411 immunoassay analyzer.

Ultrasonography and/or magnetic resonance imaging was done to look for Mullerian structures, Wolffian derivatives, and gonads. Laparoscopy/exploratory laparotomy and genitoscopy/genitogram were done when required. Diagnosis of ovotesticular DSD was confirmed by histopathological examination of the gonads. A 20 cell Karyotype was done in the patients using peripheral blood leucocytes grown in laboratory cultures and monitored till they entered in metaphase. The cells were arrested in metaphase and stained with Giemsa and viewed under microscope.

Results

In total, 48 patients who satisfied the inclusion criteria were included in the study. The age of presentation in our series varied from neonatal period to 32 years. The commonest presentation was ambiguous genitalia followed by hypospadias [Table 1]. A history of consanguinity was present in 13.4% of patients.

Androgen biosynthetic defect was the most commonly encountered diagnosis, with 5α- reductase deficiency contributing to the highest number of patients (35.4%) along with 6.2% cases of 17β- hydroxysteroid dehydrogenase deficiency [Table 2].

The characteristics of the patients with 5α-reductase deficiency is shown in Table 3. One patient in this group with ambiguous genitalia also had an anorectal malformation with imperforate anus. Genitogram was done for this patient along with barium

Table 1: Presenting complaints (*n*=48)

Variables	*n* (%)
Presenting complaints	
Ambiguous genitalia	28 (58.3%)
Hypospadias	16 (33.3%)
Primary amenorrhoea, sexual infantilism	02 (4.1%)
Primary amenorrhoea with normal female phenotype	01 (2%)
Bilateral absence of testis, no secondary sexual characters	01 (2%)

Table 2: Aetiological diagnosis (*n*=48)

Diagnosis	*n* (%)
Diagnosis	
5α-reductase deficiency	17 (35.4%)
17β-hydroxysteroid dehydrogenase deficiency	3 (6.2%)
Androgen insensitivity syndrome (AIS)	15 (31.2%)
Aetiology unclear (isolated proximal and midshaft hypospadias)	8 (16.7%)
Swyer syndrome	2 (4.2%)
Partial gonadal dysgenesis	1 (2%)
Ovotesticular DSD	1 (2%)
Vanishing testes syndrome	1 (2%)

enema, which showed urethra separate from anal canal. Anal opening was ectopic through a fistulous structure with low anorectal malformation. Only one patient of this group aged 1.2 yrs was reared as female who had a 2.5 cm phallus structure with bifid scrotal sac and a blind vaginal pouch. Gonads were palpable on the right side at superficial inguinal ring and on the left side in the scrotal sac. Patient had the urethral opening at the base of phallus-like structure (penoscrotal) with EMS of 5.5.

Three patients were diagnosed with 17β-hydroxysteroid dehydrogenase (17β HSD) deficiency one of whom presented at the age of 22 yrs with genital ambiguity and was reared as female. Patient had a SPL of 6 cm with well-formed glans and ventral chordae, perineal single opening with gonads palpable bilaterally in the labioscrotal folds, which were not fused. EMS was 3 at presentation, and there was no gynecomastia and Tanner stage was 5. Ultrasonography visualized wolffian structures without any mullerian structures. Post HCG stimulation T/A ratio was 0.4. The patient was not satisfied with the sex of rearing and desired gender reassignment. Following the diagnosis and counselling patient underwent staged surgery for genital ambiguity followed by testosterone therapy and was rehabilitated as male with satisfaction towards his new gender role.

The other two patients presented at the age of 45 days following their ambiguous genitalia noticed by parents at birth. The EMS was 3 in both patients with microphallus and post HCG stimulated T/A ratio of 0.4 & 0.3.

AIS contributed to 31.2% of cases in this series. The age range in the 15 AIS patients varied from 25 days to 32 yrs [Table 4]. Ten patients presented with complaints of ambiguous genitalia, whereas four presented with hypospadias. One patient of CAIS presented at the age of 32 years with normal female external genitalia with primary amenorrhoea, normal breast development, and sparse pubic hair. Gonads were not palpable in this patient, and ultrasound of pelvis and abdomen also failed to localize testis as well as any mullerian structures. Basal testosterone was 3.7 ng/ml with high gonadotropins and AMH value (>23 ng/ml). MRI pelvis and abdomen revealed gonadal tissue in the abdomen. Patient underwent gonadectomy, and on histopathology, the gonadal tissue was testicular. Ten patients were reared as males and five as females. There were total five patients in the peripubertal and adult age. All these patients were satisfied regarding the sex of their rearing.

The average EMS score for the AIS patients (excluding the CAIS patient who had normal female genitalia) was 5.1 ± 2.8 and that for SPL was 4.5 ± 2.9 cm. The mean AMH level was 11 ± 7.9 ng/ml, which was on the higher side.

Eight patients in this series were categorized as "etiology unclear [Table 5]" with isolated proximal and midsaft hypospadias with five having penoscrotal hypospadias, two with midshaft and one perineal hypospadias. No definite biochemical diagnosis could be established. Patients ranged from 1.5 to 18 yrs in this group. All patients were reared as males with mean EMS of 9.8 ± 2.9.

Two patients, reared as females, were diagnosed as 46 XY CGD (Swyer syndrome) presented with complaints of primary amenorrhoea with lack of secondary sexual characters. One patient was 22 yrs old, while the other 35

Table 3: Characteristics of patients with 5α-reductase deficiency

Variables	Cases (*n*=17)
Age, yrs (Mean±SD)	3.14±1.8 range: 0.25-11 yrs
Presenting complaints	
Ambiguous genitalia	11 (64.7%)
Hypospadias	6 (35.3%)
Anorectal malformation (with ambiguous genitalia)	1
Site of urethral opening	
Perineal	7 (41.2%)
Penoscrotal	5 (29.4%)
Midshaft	3 (17.6%)
Distal penile	2 (11.8%)
Sex of rearing	
Male	16 (94.1%)
Female	1 (5.9%)
EMS (External masculinisation score) (Mean±SD)	6.6±2.9
SPL; cm (Stretched penile length)	3±0.8
Testosterone; ng/ml (basal, Mean±SD)	0.3±0.02
Testosterone; ng/ml (post hcg)	2.8±2.5
T/DHT ratio (Mean±SD)	31.7±17.8
LH; mIU/ml (Mean±SD)	1.4±1.9
FSH; mIU/ml	2.9±3.6

Table 4: Characteristics of patients with AIS

Variables	Cases (*n*=15)
Age; yrs (mean±SD)	9.9±10.1 range: 25 days-32 yrs
Presenting complaints	
Ambiguous genitalia	10 (64.3%)
Hypospadias	4 (28.6%)
Primary amenorrhoea, sexual infantilism	1 (7.1%)
Sites of urethral opening	
Perineal	7 (50%)
Penoscrotal	7 (42.8%)
Normal female opening	1 (7.1%)
Sex of rearing	
Male	10 (64.3%)
Female	5 (35.7%)
Testosterone, basal; ng/ml (Mean±SD)	
Prepubertal, *n*=10 (range <0.02-0.21 ng/ml)	0.64±0.69
Adults, *n*=5 (range 2.8-8 ng/ml)	3.5±1.2
Testosterone, post hCG; ng/ml (Mean±SD)	4.3±2.4
LH; mIU/ml (Normal range: 1.7-8 mIU/ml)	
Prepubertal, *n*=10 (range <0.01-1.59 mIU/ml)	8.7±10.5
Adults, *n*=5 (range 1.7-8 mIU/ml)	27.5±22.1
AMH; ng/ml	11±7.9

Table 5: Characteristics of patients with etiology unclear (isolated proximal and midshaft hypospadias)

Variables	Cases ($n=8$)
Age; yrs (mean±SD)	9.92±7.8
	Range 1.5-18 yrs
Sites of urethral opening	
Perineal	1 (12.5%)
Penoscrotal	5 (62.5%)
Midshaft	2 (25%)
EMS (External masculinisation score) (Mean±SD)	9.8±2.9
SPL; cm (Stretched penile length) (Mean±SD)	7±2.72
Testosterone; ng/ml (basal, Mean±SD)	2.09±1.6
Testosterone; ng/ml (post hcg, Mean±SD)	4.3±2.5
LH; mIU/ml	2.5±1.9
FSH; mIU/ml	2.6±2.1
AMH; ng/ml	2.6±6.4

yrs old. Both the patients had normal female genitalia, high gonadotropin levels, and low-baseline testosterone values. Ultrasound of pelvis and whole abdomen revealed small uterus, bilateral streak gonads, and nonvisualization of wolffian structures.

One patient was diagnosed as *PGD* that presented at the age of 13 yrs with ambiguous genitalia and was reared as female. Gender role and identity was female for the patient. Patient had a small-sized (2 cm) phallus-like structure, nonpalpable gonads in labioscrotal folds with perineal urethral opening and a blind vaginal pouch. Tanners staging was prepubertal. Patient's basal testosterone was 0.5 ng/ml with LH and FSH of 9 and 12 mIU/ml, respectively. Post HCG stimulation testosterone was 2.5 ng/ml. Ultrasound scan revealed rudimentary uterine structure, streak right gonadal structure, and left gonad not visualized. Laparoscopy was done which revealed left-sided intraabdominal gonadal tissue; biopsy from which revealed testicular tissue, and subsequently gonadectomy was performed.

One patient was diagnosed as *Ovotesticular DSD* [Figure 1] who had a karyotype of 46XX/46XY and presented at an age of 11 yrs with genital ambiguity reared as male. Gender role and identity were male. The patient was prepubertal, had a poorly developed scrotal sac that was partially fused, and no gonads were palpable. There was micropenis (4 cm) with distal penile hypospadias. Basal testosterone was 0.01 ng/ml with LH of 0.34 mIU/ml and FSH of 3.4 mIU/ml. Post HCG stimulation testosterone was 1.6 ng/ml. Rudimentary uterus and vagina, no prostate, and bilateral small gonads were found on ultrasonography. Laparoscopy revealed right-sided testicular tissue and left-sided ovotestes.

One patient was diagnosed as *Vanishing testis syndrome* who presented at an age of 15 yrs with absence of testis bilaterally in the scrotal sac with nondevelopment of secondary sexual characters. Patient was reared as male, and gender role and identity was male. The patient had a fused but poorly developed empty scrotal sac and microphallus (SPL-6 cm). Patient's basal testosterone was 0.12/ml, and post HCG stimulation testosterone was 0.14 ng/ml with high LH and FSH of 30.8 and 60.3 mIU/ml, respectively. We subjected the patient to Inhibin B and AMH assays that were both low (Inhibin B: <0.1 pg/ml, AMH <0.1 ng/ml). MRI abdomen and pelvis failed to visualize testicular structure as well as prostate or seminal vesicles. Mullerian structures were also not visualized.

In total, 15 patients who were categorized as AIS along with 12 patients of isolated hypospadias were further subjected to AR gene mutation study after DNA extraction. The primers were designed against AR gene exons 3, 5, and 7. The sequencing data were obtained from the service provider, analyzed with its Blast search result, and observed the findings using BioEdit software. No mutation was found in the samples of the patients subjected to the mutation analysis.

Discussion

This study of 48 patients with characteristics described previously was carried out to get a clinical as well as hormonal spectrum of 46XY DSD in this part of the country along with some insight regarding genetics of suspected AIS patients. Highest number of patients presented with ambiguous genitalia noticed at birth (58.3%) followed by hypospadias (33.3%). In total, 83.3% of the patients were reared as males, while 16.7% were reared as females. These data are consistent with the previously reported case series.[10-13] Androgen biosynthetic defect was the most commonly encountered diagnosis followed by AIS. Patients who were designated as AIS were the second commonest etiological group in our study. However, the typical hormonal profile of high-basal LH and high-basal testosterone were not seen in all patients especially in the prepubertal group. Here, a detailed genetic study of the AR gene is required which was however not done and is a major limitation of our study. This study showed similar results with the study by R Walia *et al*.[14] and also consistent with other smaller published case series.[15-17] In our study of 17 patients with 5α-reductase deficiency, all except one were reared as males. In the study by R Walia *et al*.,[14] three patients were reared as females and they had clitoromegaly detected prepubertally. There was significant virilization during puberty in these patients and also change of gender identity to male. In another study by Aruchi Gangaher *et al*.[17], there were three 5α- reductase deficiency patients, and all three were reared as females, but male gender was reassigned in all the three patients later. The mean EMS was 6.6 ± 2.9 and mean T/DHT ratio was 31.7 ± 17.8 in our study. In Chauhan Vasundhera *et al*.[11] study, all the patients were reared as males with an average EMS of 5.1 ± 3. The mean T/DHT ratio was 21.1.

We had 15 patients of AIS where 64.3% presented with ambiguous genitalia and 28.6% with hypospadias (commonly proximal). In total, 64.3% cases were reared as males and rest as females. There were total five patients in the peripubertal and adult age. All these patients were satisfied regarding the sex of their rearing. Mean AMH was high in this group of patients

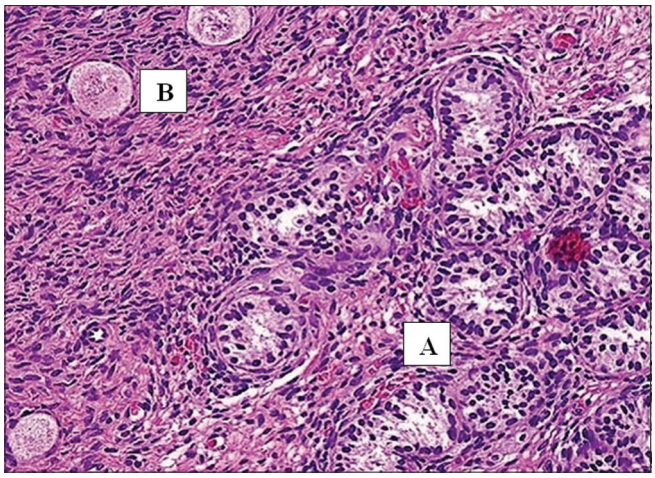

Figure 1: Ovotestes showing primitive seminiferous tubules (A) and ovarian stroma with primitive primordial follicles (B)

Figure 2: Representational picture showing no mutation in AR gene of one of the samples. (a) denotes the BLAST search result showing no mutation. (b) denotes the wild type sequence of AR gene (exon 5) and (c) denotes the query sequence which is similar to the wild type one, i.e., no mutation is found with this respect

(11 ± 7.9 ng/ml). AMH is negatively regulated by testosterone not only at puberty but also during the postnatal period. An elevation of serum AMH appears to be an interesting marker of androgen resistance or defect of androgen production in sexually ambiguous male infants as shown in the study by Rodolfo Rey *et al*.,[18] where they have shown similar trends in 20 patients.

In the present study, we had eight patients with isolated proximal and midshaft hypospadias categorized as "etiology unclear". All patients were reared as males with mean EMS of 9.8 ± 2.9 with lower mean AMH values.

In our study, there were three patients diagnosed with 17β-hydroxysteroid dehydrogenase deficiency reared as females. Significant virilization was seen in one patient presented at 22 yrs with dissatisfaction towards the sex of rearing. Patient subsequently underwent counceling and male gender reassignment and surgery. Carla Cristina Telles de Sousa Castro *et al*.[19] reported four patients with 17-β-HSD3 deficiency, showing different degrees of genital ambiguity and testosterone to androstenedione ratio <0.8 and raised as female, and female gender identity was maintained in all of them. In another study by Annemie L. M. Boehmer *et al*.,[20] 23 patients with 17βHSD deficiency were initially raised as girls.

The issue of gender assignment and sex of rearing becomes particularly challenging in certain DSDs. Unlike 5-α reductase deficiency and 17β HSD deficiency, which are bound to virilize at puberty and hence a male sex assignment is generally preferred, patients with PAIS and PGD present a challenge to the treating physician and the family. Prepubertal androgen exposure has effects on human gender behavior; those without fetal exposure to androgens like CAIS or CGD have a female phenotype, are assigned female gender, and maintain their gender into adulthood. Factors to be considered include probable adult gender identity, anticipated sexual functions, surgical options, fertility potentials, risk of gonadal malignancy and familial, social and cultural factors.[21] For patients with PAIS, a male assignment may be considered in patients with phallic growth in response to testosterone therapy.

In our study, AR gene mutation analysis designed against specific exons did not yield any results [Figure 2]. When AIS is suspected but gene exon coding is normal, flanking region sequencing, whole genome sequencing, and promoter region sequencing may reveal pathogenic variants. Variations in other genes regulating AR pathway may also be candidates to be studied.[22]

Conclusion

46 XY DSD is a heterogeneous group of disorders with a wide spectrum of clinical presentations. Elucidating the exact cause is often cumbersome and specially challenging in a resource constrained setup. Also, late presentation as well as socio-economic hindrances play a major part in the evaluation of 46 XY DSD and DSD as a whole. However, identification of an underlying cause can help with treating any coexisting hormone deficiencies and can help with anticipating any other immediate or long-term health concerns including fertility, cancer risk, and psycho-social development. The major challenge remains in identifying the appropriate gender identity and gender role concordant with normal psycho-sexual development during and after puberty. Identifying the genetic basis of any disease is always fascinating in this modern era of medical science. If resources permit, whole exome and genome sequencing represent new molecular techniques that are in the developmental stage of transition from a research tool to routine clinical diagnostic procedures.

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Conflicts of interest
There are no conflicts of interest.

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