



## Original Article

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## Etiological Classification of Patients with Ambiguous Genitalia: A Cross-Sectional Study

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### ABSTRACT

**Background:** Disorder of sex development (DSD) is one of the most serious problems encountered at birth. DSD is a heterogeneous group of rare conditions wherein chromosomal, gonadal, or anatomical sex is atypical. The aim of this study was to define the classification and etiological distribution of patients presented with ambiguous genitalia

**Methods:** This study was a cross-sectional study conducted in the department of Pediatric and Endocrinology and Surgery at Shahid Sadoughi Hospital in Yazd from March 2016 to March 2020. All patients with genital ambiguity were included in the study with the exception of five patients since they did not have a final diagnosis of ambiguous genitalia.

**Results:** Out of 50 patients, thirty-one (62 %) patients were diagnosed with 46, XXDSD, fifteen (30%) patients with 46, XYDSD, while four (8%) patients with ovotesticular DSD. Congenital adrenal hyperplasia (CAH) was the common cause in 46, XXDSD with majority due to deficiency of 21 hydroxylase enzyme. Three (6%) patients with 46, XXDSD were diagnosed with genital ambiguity as part of syndrome. Out of patients with 46, XYDSD, CAH observed in two (4%) patients, severe hypospadias in nine (18%) patients and 5-alpha reductase deficiency in two (4%) patients and one (2%) patient with syndrome. Four (8%) patients were diagnosed with ovotesticular DSD.

**Conclusion:** Our study revealed that 46, XXDSD was the most frequent DSD etiological diagnosis. CAH was the commonest cause in 46, XXDSD and severe hypospadias were the underlying cause of 46, XYDSD.

## Introduction

**D**isorder of sex development (DSD) is one of the most serious problems encountered at birth. DSD is a heterogeneous group of rare conditions wherein chromosomal, gonadal, or anatomical sex is atypical.<sup>1</sup> There is only limited information about the exact incidence and prevalence of ambiguous genitalia. The previous studies have shown that the incidence of DSD was approximately estimated one in 4500 newborn.<sup>2</sup> The prevalence of ambiguous genitalia in Iran was 0.13%.<sup>3</sup> It is very important that all efforts have been made to figure out the underlying cause of ambiguous genitalia in a child, as this can inflict a lot of concern for the parents so early sex determination may help resolve these problems.<sup>4</sup> The most common cause of ambiguous genitalia is congenital adrenal hyperplasia (CAH).<sup>5,6</sup> The etiologies of DSD are categorized into three groups: 46,XX DSD, 46,XY DSD and ovotesticular DSD.<sup>7</sup> There is a wide spectrum of conditions in 46,XY DSD such as genetic variations, altered hormonal secretion, and peripheral sensitivity to testicular hormones, which adversely affect the development of the male fetus, resulting in varying degrees of under-virilization.<sup>8,9</sup> In 46,XX DSD, the underlying cause found is congenital adrenal dysplasia (CAH), affecting 1 in 14,000-15,000 infants.<sup>10</sup>

Clinical presentation in DSD can vary. In some cases, patients are diagnosed with obvious genital ambiguity at birth. But in others, genitalia are overtly male or female with atypical internal reproductive structure. The identification of this forms of DSD may be delayed until puberty and adulthood.<sup>11</sup> Pertaining to difficulties of diagnosis and treatment of these disorders, management of intersex patients is extremely complicated, requiring coordinated team in specialized center.<sup>10</sup> Early sex assignment of children with ambiguous genitalia can help the patients and their families to deal with these problem.<sup>4</sup> Establishing a diagnosis of patients with DSD

requires adequate tools. The first test for exact diagnosis is via the genomic technologies.<sup>12</sup> With Significant progress in the understanding of the genetic causes of such disorders,<sup>13</sup> molecular diagnosis has become increasingly important.<sup>14</sup>

The aim of this study is to determine the etiological distribution, the underlying cause of ambiguous genitalia in patients observed over a four-year period (2016-2020) at Shahid Sadoughi Hospital in Yazd. We hope that the result of this study will be used to render information which will in turn optimize the standards of medical care given to patients with ambiguous genitalia and also improve the data availability on DSD, paving the way for future research.

## Materials and Methods

In this cross-sectional study, we reviewed 55 the records of patients with DSD who attended the department of Pediatric Endocrinology and Surgery at Shahid Sadoughi Hospital in Yazd from March 2016 to March 2020. The present study included 55 patients. Five patients were excluded because they did not have a final diagnosis of genital ambiguity. We retrospectively reviewed the medical records of patients which includes karyotyping, gender, age during surgery, family history of genital ambiguity, electrolyte measurements, external genital anatomy, antenatal history, radiological evaluation (ultrasound or magnetic resonance imaging) and hormonal analysis including cortisol, testosterone (T), estradiol (E2), dehydrotestosterone (DHT), cortisol, adrenocorticotropic hormone (ACTH), follicle stimulating hormone (FSH), luteinizing hormone (LH), and 17-hydroxyprogesterone (17-OHP). Moreover, in some cases, the relevant data were obtained from the patient's records of gonadal biopsy, laparoscopy, SRY gene, histopathological result, the level of mullerian hormone and inhibin B. In addition, if the records were incomplete, we contacted the family to request for copies of medical files. We also searched for

patients with any anomalies in those with multiorgan damages. Diagnosis of ovotesticular DSD and gonadal dysgenesis were confirmed by surgical history and histopathological reports. All the patients were evaluated by a team composed of pediatric surgeon, pediatric endocrinologist, neonatologist, pediatric urologist and pathologist.

**Ethical considerations:** Each participant in the study was informed of the study's purpose and provided informed consent before participation in the study. The study protocol has been approved by Shahid Sadoughi University of Medical Sciences' ethics committee, Yazd, Iran. (Ethical Code: IR.SSU.SPH.REC.1400.097).

**Statistical analysis:** Statistical analyses were conducted with Statistical Package for the Social Sciences (SPSS version 20.1 for Windows; SPSS Inc., Chicago, USA). As appropriate, the data are presented in numbers, Mean  $\pm$  SD or median. Chi-square test was used and for comparing continuous variables among the groups. Frequency and percentage were used for categorical variables.

## Results

In total, we included 50 individuals with any degree of gonadal ambiguity. The mean age of patients at presentation was 62 months with a median of 48 months ranging from two days to twenty years. The mean age of surgery was 12 months. In terms of karyotype analysis, they were classified into 31 (62%) patients with 46, XXDSD, 15 (30%) patients with 46, XYDSD and 4 (8%) with ovotesticular DSD.

46, XXDSD was the most frequent etiology in our study (n = 31).

The classification of the patients is depicted in Table 1. The commonest diagnosis among all the patients in this study was CAH.

The majority of patients with 46, XXDSD were diagnosed with CAH, comprising 22 (71%) patients with 21-hydroxylase deficiency (the type of 21-hydroxylase deficiency, salt or non-salt wasting, did not mention due to incomplete information of 2-3 patients' files), six (19.3%) patients with 11-beta-hydroxylase deficiency. The most common cause of 46, XYDSD was severe hypospadias in nine (60%) patients. A 46XY karyotype was found in all of the patients with severe hypospadias. Accordingly, CAH was found in two (13.3%) patients with 46, XYDSD, these patients had 3-beta hydroxylase deficiency.

The 5-alpha-reductase deficiency was found in two (13.3%) patients with 46, XYDSD. One patient (6.7%) was diagnosed to have Persistent Mullerian duct syndrome with 46, XYDSD. Among them, four (8%) patients were diagnosed with ovotesticular DSD.

Four (8%) patients had ambiguity as part of the syndrome while one case had 46, XXDSD with trisomy 13 and imperforated anus, another case had 46, XXDSD with imperforated anus and choanal atresia, and the other one had 46, XXDSD with ambiguous genitalia associated with imperforated anus who died in the neonatal period. These patients had multiorgan damages with normal hormonal measurements. The detailed etiology of DSD is displayed in Table 2.

**Table 1.** Etiological Classification of Disorders of Sex Development (DSD) in the Study Population (n = 34)

Etiological classification		Etiological diagnosis	N (%)
46,XXDSD	N = 31 (62%)	Congenital adrenal hyperplasia	28 (56%)
		Syndromic	3 (6%)
46,XYDSD	N = 15 (30%)	Congenital adrenal hyperplasia	2 (4%)
		Syndromic	1 (2%)
		Persistent Müllerian duct syndrome	1 (2%)
		Severe hypospadias	9 (18%)
		5 alpha reductase deficiency	2 (4%)
Ovotesticular DSD (True Hermaphroditism)	N =4 (8%)		4 (8%)

**Table 2.** Etiological Classification and Diagnosis of Patients

Classification	Diagnosis	Frequency (n)	Percent (%)
46,XXDSD	Congenital adrenal hyperplasia	28	90.3%
	21-hydroxylase deficiency	22	71.0%
	11-beta-hydroxylase deficiency	6	19.3%
46,XYDSD	Syndromic	3	9.7%
	Congenital adrenal hyperplasia	2	13.3%
	3-beta hydroxylase deficiency	2	13.3%
	Syndromic	1	6.7%
	5 alpha reductase deficiency	2	13.3%
	Persistent Mullerian Duct	1	6.7%
True hermaphroditism (ovotesticular DSD)	Severe hypospadias	9	60.0%
		4	100/0%

\*Chi-square test was used.

### Discussion

In this study, we presented 50 diagnosed cases over four years. Majority (61.7%) of the patients presented before the age of 5 years.

Only two patients with ambiguous genitalia who had 46XX karyotype because of opinion of their parents and psychiatrist underwent surgery and change to male phenotypes. Our data determined the most common cause of DSD was 46, XXDSD (62%), with 46, XYDSD in second place (30%) and lastly ovotesticular disorders (8%).

In contrast, 46, XYDSD was the commonest type of DSD in other reports.<sup>15-17</sup> The etiological classification of the patients is portrayed in Table 1.

46, XXDSD were the most common cause of DSD in our study, with CAH as the underlying diagnosis. These findings were consistent with the result of studies which reported that CAH is the most common cause of DSD.<sup>11,15,18,19</sup> However Ganie et al<sup>20</sup> found the most common diagnosis was unclassified disorder of androgen synthesis and action (53%) and ovotesticular DSD (22%) in South Africa. Erdoğ an and their co-authors<sup>6</sup> reported the most common cause of DSD were Turner Syndrome and CAH. However, we had not found any cases with Turner Syndrome in this study because we only observed the patients who attended the Shahid Sadoughi Hospital in Yazd.

Among 31 patients with 46, XXDSD, we found that most of the individuals with CAH

had deficiency of 21-hydroxylase enzyme. These results were consistent with other studies.<sup>(16, 21-23)</sup> Six patients (19.3%) with 46, XXDSD had 11 beta deficiency. It is important to diagnose these children early so that they can receive treatment in order to avoid severe virilization, hirsutism, short stature, and premature pubarche.<sup>24</sup>

The most common cause of DSD in 46, XYDSD group was severe hypospadias<sup>15,17</sup> (60%) with undescended testis (UDT). Also, the patients with severe hypospadias showed normal hormonal evaluation. Several researches have reported the most common cause in this group to be androgen insensitivity syndrome.<sup>9,15</sup> Misgar and their co-workers<sup>22</sup> reported the most common cause of the 46,XYDSD were 5-alpha-reductase deficiency. In our study, two children with 46, XYDSD had 5alpha reductase. Accordingly, two children (13.3%) with CAH in this group had 3-beta hydroxylase deficiency. Studies reported ovotesticular DSD were rare.<sup>20,25</sup> The constitution of ovotesticular DSD is 3%-10% of all sexual disorders.<sup>25</sup> Level of genital ambiguity in patients with ovotesticular DSD was variable. Three patients in our study with ovotesticular DSD had 46XX karyotype and one patient had 46XY karyotype. These findings were similar to previous studies reporting 72% patients with 46XX karyotype.<sup>15</sup>

### Conclusion

In conclusion, the most common cause of

DSD found in this study is CAH. Meanwhile, 46, XX DSD was more common than 46, XYDSD. In 46, XXDSD, the underlying cause was CAH and the common cause of DSD in 46, XYDSD was severe hypospadias. This cross-sectional study conducted at a single center acted as a major limiting factor with our evaluation including a small number of patients. However multicenter studies with a lifelong follow-up are required to find the precise prevalence of patients with DSD in our population. There is a further need for genetic test in patients with ambiguous genitalia with syndromes.

### Conflict of Interests

Authors have no conflict of interests.

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