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Original Research Article

Clinical Spectrum of Disorders of Sexual Differentiation in Children

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Abstract: *Disorders of sexual differentiation (DSD) are a heterogeneous group of congenital conditions characterized by atypical development of chromosomal, gonadal, or anatomical sex.* ***Objective:*** *To evaluate the clinical spectrum, modes of presentation, and underlying etiologies of DSD in children presenting with ambiguous genitalia.* ***Methods:*** *This cross-sectional study was conducted at the Department of Pediatrics, Rawalpindi Medical University (RMU) and Allied Hospitals from August 2023 to July 2024. A total of 110 children aged from birth to 12 years with ambiguous genitalia were enrolled using non-probability consecutive sampling. Data were collected using a predesigned proforma, including detailed history, physical examination, External Masculinization Score (EMS), and Prader staging for 46 XX cases.* ***Results:*** *The mean age at presentation was 3.8 ± 2.9 years, with 62.7% presenting in infancy. Ambiguous genitalia at birth was the most common mode of presentation (65.5%), followed by inguinal/labial swellings (16.4%). Among 46 XY patients (n=64), androgen insensitivity syndrome (28.1%) and 5-α reductase deficiency (18.8%) were the most frequent causes. In 46 XX patients (n=46), congenital adrenal hyperplasia was predominant (73.9%), with ovotesticular DSD accounting for 17.4%. Most 46 XX patients exhibited moderate to severe virilization (Prader stages III–IV). The mean EMS across all patients was 5.4 ± 2.3, consistent with the presence of genital ambiguity.* ***Conclusion:*** *DSD in children most commonly presents at birth with ambiguous genitalia, with AIS and CAH being the leading etiologies among 46 XY and 46 XX patients, respectively. The high proportion of severe genital ambiguity reflects delays in referral and Diagnosis.*

Keywords: Disorders of sexual differentiation, ambiguous genitalia, congenital adrenal hyperplasia, androgen insensitivity syndrome

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Introduction

Disorder of sex development (DSD) is defined as a congenital condition in which the development of chromosomal, gonadal, or anatomic sex is atypical. The incidence of DSD is approximately 1 in 4,500 to 1 in 5,000 live births (1). It is a social emergency as the decision-making in relation to sex assignment has been perceived as extremely disturbing and challenging to both families and healthcare professionals. Normal sexual development in utero is dependent upon a precise and coordinated sequence of events that involves various activating and repressing factors (2). The phenotypic manifestations of DSDs are diverse and can include bilateral undescended testes, severe hypospadias (scrotal or perineal), clitoromegaly, fusion of the posterior labial folds, female external genitalia with palpable gonads, discordant genitalia, and sex chromosome abnormalities (3). The term "disorders of sex differentiation" replaced previously used terms like ambiguous genitalia or intersex disorders as a result of a new classification proposed during the Chicago Consensus meeting held in 2006. Another expert consensus document was proposed in 2018, recommending the term "differences of sex development" and following a classification similar to that of Chicago, also based on karyotype (4).

The development of sexual characteristics in humans is a tightly regulated process involving sequential stages: chromosomal sex determination at fertilization, gonadal differentiation, and phenotypic sex development through the action of hormones. Disturbances at any stage may lead to DSD. Etiological categories include sex chromosome abnormalities (such as Turner syndrome or Klinefelter syndrome), disorders of gonadal differentiation (gonadal dysgenesis), and defects in androgen synthesis or action (as in congenital adrenal hyperplasia or androgen insensitivity syndrome) (5). Each category has distinct clinical and biochemical profiles, yet overlapping phenotypes are not uncommon, complicating the diagnostic process. Clinically, the presentation of DSD varies depending on the underlying pathology and the age at Diagnosis (6). Neonates often present with ambiguous genitalia, which remains the most striking and distressing feature for families and healthcare providers. In childhood, patients may be identified through the evaluation of inguinal hernias, atypical pubertal changes, or failure to exhibit expected growth patterns (7). Adolescents, particularly females, may present with primary amenorrhea, virilization, or underdeveloped secondary sexual characteristics. Notably, some cases remain undetected until adulthood, underscoring the heterogeneous nature of these disorders (8).

In boys with atypical genitalia, partial or complete androgen insensitivity, 5-α reductase deficiency, and congenital adrenal hyperplasia are common underlying etiologies. Congenital adrenal hyperplasia (CAH) is the most common cause of virilization in females, mainly presenting with clitoromegaly and signs of androgen excess (9). It is anticipated that 33.7% of the DSD patients have genital anomalies. Disorders of sexual differentiation are poorly explored in Pakistan due to the stigma associated with them. Minimal studies have been conducted in our setup (10). The study is specifically needed for a deeper understanding of patterns of DSD in a resource-constrained tertiary care setting. This will help pediatricians diagnose such conditions promptly. Early Diagnosis and timely referral to pediatric endocrinologists can help avoid unnecessary delays and errors in determining the sex of rearing in these children (11).

The basic aim of the study is to study the frequency of genital anomalies, the mode of presentation, and the probable cause of genital anomalies in patients with Disorders of Sexual Differentiation.

Methodology

This Cross-Sectional Study was conducted at RMU and Allied Hospitals, Department of Pediatrics, Rawalpindi Medical University (RMU), from August 2023 to July 2024. The sample size was calculated using the World Health Organization (WHO) sample size calculator. The following parameters were applied: Confidence level: 95% Margin of error: 9% anticipated population rate of genital anomalies in children with disorders of sexual differentiation (DSD): 33.7%

Based on these assumptions, the calculated sample size was 110 patients. Non-probability consecutive sampling was employed to recruit eligible participants who presented during the study period, specifically children aged 0 to 12 years. Patients with ambiguous genitalia are defined as having external genitalia that do not have typical male or female characteristics. Children whose parents or guardians declined consent to participate in the study were excluded. The study was initiated after obtaining ethical approval from the College of Physicians and Surgeons. Written informed consent was obtained from parents or legal guardians of all participants prior to enrollment. A predesigned proforma was used for uniform data collection. Each child underwent a detailed clinical evaluation by pediatricians with a special interest in pediatric endocrinology, as well as the principal investigator. A comprehensive history was obtained with particular emphasis on features of genital ambiguity, family history, and any prior interventions. A thorough physical examination was performed, with special attention to genital anatomy. To standardize assessment, the External Masculinization Score (EMS) was calculated for all patients, with scores ranging from 0 to 12; values below seven were considered ambiguous. In phenotypic females, the degree of virilization was assessed using the Prader scale, which classifies genital appearance into five stages, ranging from isolated clitoromegaly without labial fusion (Stage I) to a penile clitoris with urethral meatus at the tip and scrotum-like labia (Stage V). Potential etiologies were evaluated for both 46 XY DSD (including androgen insensitivity syndrome, 5-α reductase deficiency, congenital adrenal hyperplasia, primary testicular failure, ovotesticular DSD, and Klinefelter syndrome) and 46 XX DSD (including congenital adrenal hyperplasia and ovotesticular DSD). Data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 23. Qualitative variables, such as the type of genital anomaly, mode of presentation, Prader stage, and underlying causes of DSD, were expressed as frequencies and percentages. Quantitative variables, including EMS scores and age at presentation, were analyzed as mean ± standard deviation. The data were further stratified by gender, and post-stratification chi-square tests were used to determine the associations. A p-value of less than 0.05 was considered statistically significant.

Results

Data were collected from 110 patients, comprising 58 males (52.7%) and 52 females (47.3%). The overall mean age at presentation was 3.8 ± 2.9 years, with males presenting slightly later (4.2 ± 3.1 years) compared to females (3.3 ± 2.7 years), although this difference was not statistically significant ($p = 0.21$). The majority of patients, 69 (62.7%), presented within the first year of life, reflecting early parental concern when ambiguity is detected at birth. Another 28 (25.5%) presented between 2 and 5 years of age, while 13 (11.8%) were brought for evaluation between 6 and 12 years. No significant differences were noted between male and female groups across age categories ($p > 0.05$).

The most common mode of presentation was ambiguous genitalia at birth, observed in 72 children (65.5%). Inguinal or labial swellings, suggestive of undescended gonads, accounted for 18 cases (16.4%). Older children presented with delayed puberty (7.3%) or primary amenorrhea (6.4%), while recurrent urinary tract infections were reported in 5 patients (4.5%). Among the 64 children with 46 XY DSD, androgen insensitivity syndrome (AIS) was the most frequent Diagnosis, accounting for 18 cases (28.1%). This was followed by 5-α reductase deficiency (18.8%) and congenital adrenal hyperplasia (17.2%). Primary testicular failure was identified in 9 children (14.1%), ovotesticular DSD in 8 (12.5%), and Klinefelter syndrome in 6 (9.3%).

Among the 46 children with 46 XX DSD, congenital adrenal hyperplasia (CAH) was the overwhelmingly predominant cause, diagnosed in 34 patients (73.9%). Ovotesticular DSD was identified in 8 children (17.4%), while 4 cases (8.7%) were categorized as other or unclassified. Stage III was the most common, observed in 12 patients (26.1%), followed by Stage IV in 11 (23.9%). Ten children (21.7%) were classified as Stage II, while milder forms (Stage I) accounted for 7 cases (15.2%). Complete masculinization (Stage V) was seen in 6 patients (13.0%).

The mean EMS for the entire cohort was 5.4 ± 2.3, consistent with ambiguous genitalia (EMS <7). Males had a slightly higher mean score (5.8 ± 2.1) compared to females (4.9 ± 2.4), but both groups remained within the ambiguous range.

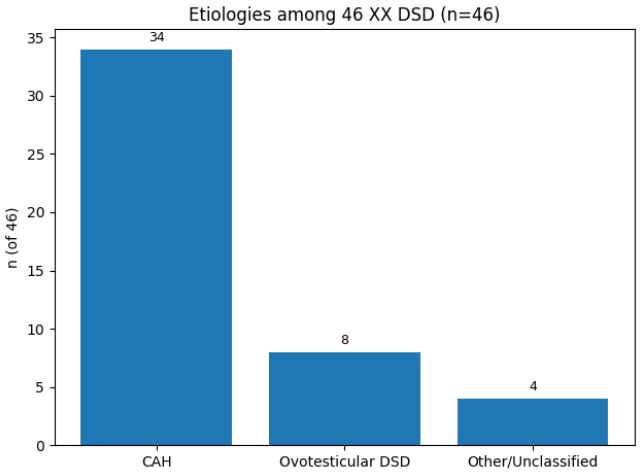

Figure 1. Etiologies among 46 XX DSD

Table 1. Demographic Characteristics of Patients (N = 110)

Variable	Total (N=110)	Male (n=58)	Female (n=52)	p-value
Age at presentation, years (mean ± SD)	3.8 ± 2.9	4.2 ± 3.1	3.3 ± 2.7	0.21
≤1 year, n (%)	69 (62.7)	36 (62.1)	33 (63.5)	0.87
2–5 years, n (%)	28 (25.5)	14 (24.1)	14 (26.9)	0.73
6–12 years, n (%)	13 (11.8)	8 (13.8)	5 (9.6)	0.49

Table 2. Modes of Presentation (N = 110)

Mode of Presentation	Frequency n (%)
Ambiguous genitalia at birth	72 (65.5)
Inguinal/labial swelling (possible gonads)	18 (16.4)
Delayed puberty	8 (7.3)
Primary amenorrhea	7 (6.4)

Recurrent urinary tract infections	5 (4.5)
Diagnosis	
Androgen insensitivity syndrome	18 (28.1)
5-α reductase deficiency	12 (18.8)
Congenital adrenal hyperplasia	11 (17.2)
Primary testicular failure	9 (14.1)
Ovotesticular DSD	8 (12.5)
Klinefelter syndrome	6 (9.3)

Table 3. Causes of 46 XX DSD (n = 46)

Diagnosis	Frequency n (%)
Congenital adrenal hyperplasia	34 (73.9)
Ovotesticular DSD	8 (17.4)
Other/Unclassified	4 (8.7)
Prader Stage	
Stage I	7 (15.2)
Stage II	10 (21.7)
Stage III	12 (26.1)
Stage IV	11 (23.9)
Stage V	6 (13.0)

Table 4. Mean External Masculinization Score (EMS) by Gender (N = 110)

Group	Mean EMS ± SD
Males (n=58)	5.8 ± 2.1
Females (n=52)	4.9 ± 2.4
Total (N=110)	5.4 ± 2.3

Discussion

This study explored the clinical spectrum of disorders of sexual differentiation (DSD) in children presenting with ambiguous genitalia at a tertiary care hospital in Pakistan. A total of 110 patients were analyzed, with most presenting within the first year of life. The findings highlight important diagnostic patterns, gender-specific variations, and causes of DSD in our setting. Early age at presentation in the majority of cases (62.7% in infancy) reflects parental concern when genital ambiguity is detected at birth. This pattern is consistent with previous research, where ambiguous genitalia remains the most frequent reason for referral to specialized centers. However, delayed presentations, including primary amenorrhea and delayed puberty, were also observed. Such late referrals likely represent limited awareness among families, social stigma, and restricted access to pediatric endocrinology services in Pakistan (12).

Among 46 XY DSD patients, androgen insensitivity syndrome (AIS) emerged as the leading cause (28.1%), followed by 5-α reductase deficiency and congenital adrenal hyperplasia. This distribution is comparable to reports from South Asian and Middle Eastern countries, where AIS remains a dominant etiology due to high rates of consanguinity and inherited defects in the androgen receptor gene. The proportion of 5-α reductase deficiency was also notable, highlighting the role of enzymatic defects in local populations (13). Gonadal dysgenesis and ovotesticular DSD were less frequent but clinically significant due to their long-term malignancy risk. In contrast, congenital adrenal hyperplasia (CAH) was overwhelmingly the most common cause of 46 XX DSD (73.9%). This finding aligns with the global literature, where CAH is the predominant etiology of virilization in genetic females. The high prevalence of CAH in our study underscores the need for neonatal screening programs, which remain absent in many low- and middle-income countries, including Pakistan. Early detection through biochemical screening could reduce morbidity and improve gender assignment decisions (14-16).

The distribution of Prader stages among 46 XX patients revealed that the majority presented with moderate to severe virilization (Stages III and IV). This finding suggests that affected families often delay medical consultation until genital ambiguity becomes more pronounced, reflecting both lack of awareness and sociocultural sensitivities surrounding genital abnormalities. Such delays contribute to psychosocial stress for families and complicate timely gender assignment (17). The mean External Masculinization Score (EMS) in our cohort was 5.4 ± 2.3, consistent with ambiguous genitalia (EMS <7). These findings highlight the utility of EMS as a standardized tool in evaluating DSD, providing an objective assessment in populations where laboratory and genetic resources may be limited (18).

From a public health perspective, the results of this study reinforce the urgent need for multidisciplinary care models in Pakistan. Pediatric endocrinologists, surgeons, geneticists, and psychologists must work collaboratively to ensure accurate Diagnosis and holistic management (19). Importantly, ethical considerations regarding gender assignment and surgical interventions require culturally sensitive but medically informed approaches. Previous research has emphasized the importance of delaying irreversible interventions until affected individuals can actively participate in decision-making. This recommendation holds particular relevance in our setting, where cultural expectations often drive early gender assignment. The findings also highlight significant gaps in healthcare delivery (20). The absence of routine neonatal screening for CAH, limited availability of advanced molecular diagnostics, and a lack of specialized multidisciplinary clinics contribute to delayed Diagnosis and suboptimal management. Addressing these deficiencies could improve outcomes and reduce long-term complications, including infertility, gonadal tumors, and psychosocial maladjustment (21). Limitations of the present study include its cross-sectional design and reliance on hospital-based cases, which may not reflect the true prevalence of DSD in the community. Genetic confirmation was not possible in all cases due to resource limitations, and long-term outcomes such as fertility and psychosocial adaptation were not assessed. Nevertheless, this study provides valuable insight into the clinical patterns of DSD in Pakistani children and establishes a foundation for larger multicenter studies.

Conclusion

It is concluded that disorders of sexual differentiation in children present with a broad clinical spectrum, with ambiguous genitalia at birth being

the most common mode of presentation. Among 46 XY DSD, androgen insensitivity syndrome and 5-α reductase deficiency were the predominant causes, while congenital adrenal hyperplasia was overwhelmingly the leading cause in 46 XX DSD. The majority of children exhibited moderate to severe genital ambiguity, highlighting delays in referral and Diagnosis. These findings underscore the need for early recognition, routine neonatal screening programs, and access to multidisciplinary care to ensure accurate Diagnosis, appropriate gender assignment, and improved long-term outcomes.

Declarations

Data Availability statement
All data generated or analysed during the study are included in the manuscript.
Ethics approval and consent to participate
Approved by the department concerned. (IRBEC-24)
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Conflict of interest

The authors declared the absence of a conflict of interest.

Author Contribution

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All authors reviewed the results and approved the final version of the manuscript. They are also accountable for the integrity of the study.

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