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# Genetic and Clinical Study of Disorders of Sex Development among Children and Adolescents

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**Abstract:** Introduction: Disorders of sex development (DSD) include any congenital condition with atypical development of chromosomal, gonadal, or anatomical sex. They are not uncommon in Egypt; genital ambiguity had an incidence of 1/5000 per 20.000 new-borns and infants. DSD are classified into 46,XY DSD, 46,XX DSD and Sex chromosome DSD. Objectives: To determine frequency of different categories of DSD among children and adolescents and to assess clinical, cytogenetic and molecular characteristics of each type. Patients and Methods: 223 patients, between 0-18 years of age, were referred to Genetic Endocrinology Clinic at NRC between 2017 and 2019 with different presenting features suggesting DSD. All patients were subjected to thorough clinical examination, chromosomal analysis and pelvic sonar. Biochemical testing, FISH analysis, molecular study and gonadal histopathology were done when indicated. Results: Patients were put into three main karyotype based groups; first group had sex chromosome DSD 49%, second group had 46,XY DSD 34% and the third group had 46, XX DSD 17%. Patients varied at age of first presentation with mean age 10.13 ± 7.24 years. Patients showed wide range of presenting complaints; the most frequent was ambiguous genitalia followed by primary amenorrhea. The most frequent causes were TS, KS, CAH and 5 alpha reductase deficiency.
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**Keywords:** DSD- Ambiguous genitalia- XY DSD- XX DSD- Sex chromosome

## 1. Introduction:

DSD include congenital conditions with atypical development of chromosomal, gonadal or anatomical sex. One of 4,500 infants is born with external genital abnormality **(24)**. In Egypt DSD are not uncommon; genital ambiguity was reported in 1/5000 per 20.000 newborns and infants **(33)**. Disorders of sex development are classified into three main categories; 46,XY DSD, 46,XX DSD and sex chromosome DSD **(24)**.

Phenotypic spectrum of DSD is widely variable; it could present early with genital ambiguity, undescended testes, hypospadias, inguinal hernia in a female, discordant genitalia and karyotype, and late may present with previously unrecognized genital ambiguity, delayed puberty, primary amenorrhea, virilization of female external genitalia, male gynecomastia **(24).**

Etiology of DSD is multifactorial and could be attributed to both genetic and environmental factors **(14)**. Understanding the genetic basis of DSD will lead to refining their diagnosis and management **(45).** Advanced molecular genetic techniques improved the DSD diagnostic capabilities but the lack of proper insurance coverage limits their access to many families **(28)**. There are only a handful of comprehensive systematic studies of etiological background as well as management plans for DSD patients **(27).**

## 2. Patients and methods:

This Cross-sectional, descriptive study included all patients with inclusion criteria suggestive of DSD according to **(24),** age spectrum from 0 to 18 years old, who were referred to Clinical Genetics and Endocrinology Clinics, Medical Research Center of Excellence (MRCE), National Research Centre (NRC) during 18 months from September 2017 to February 2019. Following written informed consent form of Research Ethics Committee in the Faculty of Postgraduate Childhood Studies, patients were subjected to 1) detailed history taking including pedigree analysis, 2) thorough clinical examination with special emphasis on genital examination, 3) cytogenetic Studies including chromosomal analysis for all patients using GTG-banding technique **(49,55),**

karyotype description followed the recommendations of ISCN **(26)** and fluorescence in situ hybridization (FISH) using commercial X and Y probes **(48)**, 4( biochemical investigations to assess serum basal and post human chorionic gonadotropin testosterone and its precursors, DHT, 17(OH) progesterone, estradiol, gonadotropins, Na, K, rennin and anti-Mullerian hormone, 5) abdomeno-pelvic ultrasound to assess internal genital organs, 6) molecular genetic studies for mutational analysis of candidate DSD genes like AR, SRD5A2 and 17BHSD; DNA extraction from peripheral blood lymphocytes using salting out technique **(40)**, DNA sequencing was performed then data were interpreted, and 8) laparoscopy and gonadal histopathological diagnosis of gonadal dysgenesis and ovotesticular DSD.

Collected data were statistically analyzed to assess the frequencies of occurrence of different types of DSD using **SPSS. V.20 (25**)

**3. Results:**

This study included 223 patients with clinical criteria suggestive of DSD as described by **(24).** Patients were classified into three main categories; sex chromosome DSD (109/223) 49%, 46,XY DSD (76/223) 34% and 46,XX DSD (38/223) 17%. Studied patients varied in age at first presentation with mean age 10.13 ± 7.24years, correlation of category of DSD and age at first presentation of studied patients is presented in **(Table 1).** Consanguinity was identified in 52% of patients; being higher in non-sex chromosome DSD. Studied patients showed wide range of presenting complaints; the most common were ambiguous genitalia 34%, primary amenorrhea 23%, short stature 12% and hypogonadism 9%. Correlation of category of DSD and presenting complaints of studied patients is presented in **(Figure 1).**

**Table (1):** Age of first presentation of studied patients with DSD

| Age in years | 46,XX | 46,XY | Sex chromosome DSD | Total |
|---|---|---|---|---|
| **0-2** | 14 | 34 | 14 | 62 |
| **3-12** | 9 | 17 | 25 | 51 |
| **13-18** | 15 | 25 | 70 | 110 |
| **Total** | 38 | 76 | 109 | 223 |
| **Mean age** | 8.53 ± 7.73 | 7.08 ± 6.95 | 12.83 ± 6.23 | 10.13 ± 7.24 |

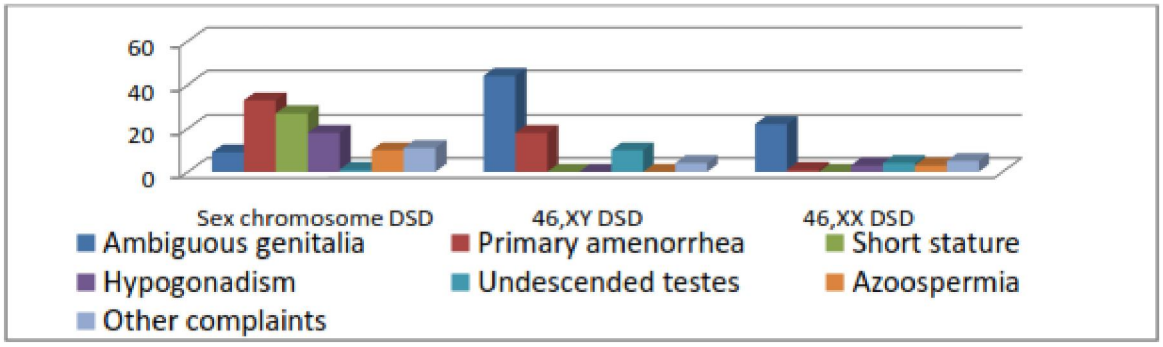


**Figure (1):** Primary complaint and Category of DSD chart

Sex chromosome DSD included 3 subcategories; the commonest was Turner syndrome (TS) (57 patients) followed by Klinefelter syndrome (KS) (36 patients) and other sex chromosome DSD (16 patients). Patients had wide range of cytogenetic abnormalities. Numerical abnormalities were detected in 78% and structural abnormalities were detected in 22%. Gonadal histopathology confirmed the diagnosis of mixed gonadal dysgenesis (MGD) in 9 patients and ovotesticular DSD in 4 patients.

46, XY DSD included 3 subcategories; the commonest were disorders of androgen synthesis or action (45 patients), followed by disorders of gonadal development (16 patients) and other 46, XY DSD (15 patients). Molecular gene mutations were detected in 35 patients; SRD5A2 gene mutation (23 patients), 17BHSD3 gene mutation (6 patients) and AR gene mutation (6 patients). 3 male reared patients who presented with undescended testes were diagnosed of having testicular regression syndrome; confirmed by imaging and laparoscopy. 12 patients had 46,XY gonadal dysgenesis confirmed by histopathology.

46, XX DSD included 3 subcategories; the commonest were disorders of androgen excess (25 patients), followed by disorders of gonadal development (11 patients) and other 46, XX DSD (2 patients). 8 male reared patients had XX testicular DSD confirmed by histopathological diagnosis.

Eight patients had histopathological diagnosis of ovotesticular DSD; 4 patients had sex chromosome DSD, 3 patients had 46,XX DSD and 1 patient had 46,XY DSD. Seventeen patients had DSD in association with other congenital anomalies; 15 of them had 46,XY DSD and 2 of them had 46,XX DSD.

**4. Discussion:**

There are few attempts to estimate frequencies of different categories of DSD according to the 2006 classification system **(24)**. In our study 49% of patients had sex chromosome DSD, 34% had 46,XY DSD and 17% had 46,XX DSD. In similar previous studies from different parts of the world 46,XY DSD was the commonest category **(19,21,27,56).**

Patients varied in age at first presentation with mean age of 10.13±7.24 years for total DSD, 7.08± 6.95 years for 46,XY DSD, 8.53± 7.73 years for 46,XX DSD and 12.83± 6.23 years for sex chromosome DSD. Similar studies had a wide variation in age at first presentation with mean age of 6.5±6.5 years for total DSD **(19)**, 2.2 ± 3.8 years for 46,XY DSD, 2.4 ± 4.6 years for 46,XX DSD and 5.3 ± 5.8 years for sex chromosome DSD **(27)**.

Parental consanguinity was detected in 52% of patients; especially among 46, XY DSD patients 70% and 46, XX DSD patients 61%. This is consistent with previous statement that consanguinity may lead to an increase in incidences of both 46,XY and 46,XX DSD **(8).**

Patients had a wide range of presenting complaints; 34% of patients presented with genital ambiguity, 23% of female reared patients presented with primary amenorrhea and 15% of male reared patients presented with hypogonadism and azoospermia. DSD patients could present early in life when ambiguous genitalia is noticed during physical examination but later presentations could be detected in childhood, adolescence and adulthood; including delayed or incomplete puberty **(29)**.

Sex chromosome DSD patients were classified into 3 subcategories; 52% of them had TS, 33% had KS and 15% had other abnormalities. Similar studies reported that TS was the most common diagnosis in patients with sex chromosome DSD **(21,27).** Numerical sex chromosome abnormalities were more frequently encountered than structural abnormalities, which is consistent with previous statement **(7)**.

Cytogenetic analysis of TS patients revealed that 24% had classic TS 45,X, 27% had 45, X in mosaicism and only 2% had TS variant 46,X,i (Xq). A previous study of Turner patients reported that 11% had classic 45,X, 78.5% had mosaicism and only 5% had 46,X,i (Xq) **(37)**. Other studies from different parts of the world reported a high observation of classic 45,X karyotype **(2,5,18,32,42)**. Despite more previous studies reporting higher frequency of 45,X karyotype than in our study; it is stated that more than 95% of 45,X embryos will not survive embryological development and those who survive to the end are often mosaic **(17)**.

Cytogenetic analysis of KS patients revealed that 28% had classic KS 47,XXY and only 4.6% had KS variants which is consistent with previous studies of the pattern of KS **(5,31,53)**.

Gonadal histopathology confirmed the diagnosis of MGD in 9 patients with sex chromosome DSD. MGD consists of a heterogeneous group with diverse phenotypic and gonadal abnormalities **(6)**, but it typically presents with a streak gonad on one side and a dysgenetic or normal appearing testis on the other side **(51)**. Female reared patients with MGD should have bilateral gonadectomy upon diagnosis because of increased risk of malignant transformation **(29,44)**.

Two female reared patients with primary amenorrhea had structurally abnormal X chromosome. Structural X chromosome aberrations including translocations, deletions, and marker chromosomes were identified in patients with primary or secondary amenorrhea **(5)**. One male reared patient with hypogonadism had 45,X with SRY translocated on an autosome. SRY gene could be translocated mostly on X chromosome but very few cases of 45,X testicular DSD with autosome translocation were published to date, the prognosis of such cases depends on the autosome involved in the translocation **(13,41)**.

46,XY DSD included 3 subcategories; disorders of androgen synthesis or action 59%, disorders of gonadal development 21% and other 46,XY DSD 20%. Previous similar studies reported disorders of androgen synthesis or action as the commonest subcategory of 46,XY DSD patients **(56,21)**. Molecular gene mutations were detected in 46% of 46,XY DSD patients. Genetic mutations could be detected in less than 50% of 46, XY DSD patients, but advanced genetic testing like whole exome sequencing helped in improving identification of genetic mutations in known as well as novel DSD genes **(3,4)**.

SRD5A2 gene mutation causing 5 alpha reductase deficiency was detected in 30% of 46,XY DSD patients. Similarly in a previous study from Egypt; the majority of 46,XY DSD patients had a diagnosis of 5 α-reductase deficiency but in another study from Sudan the commonest diagnosis was androgen insensitivity syndrome (AIS) **(1,34)**. 5 alpha reductase deficiency is thought to be rare and most of the patients have been reported in Dominican Republic, Papua New Guinea, Turkey, Mexico, Brazil, and Asia **(18,23).**

AR gene mutation causing AIS was detected in 8% of 46,XY DSD patients. In a recent Danish

nationwide study, the estimated prevalence of AIS was 2.3 per 100,000 live born females **(12)**.

17BHSD3 gene mutation causing 17 beta hydroxysteroid dehydrogenase deficiency (17BHSD) was detected in 8% of 46,XY DSD studied patients. 17BHSD deficiency represent approximately 4% of 46,XY DSD patients of the largest DSD European database, its incidence is estimated to be around 1:147,000 newborns in a study conducted in Netherlands but Just like other autosomal recessive disorders; the frequency of the disorder is increased in areas with high rate of consanguinity **(39)**.

Five male reared patients presented with undescended testes had PMDS. So far; 81 families with PMDS had different mutations of the AMH gene, 79 families had different mutations of the AMHR2 gene, and no mutation could be detected in 12% of patients **(46)**. PMDS typically present with unilateral or bilateral undescended testis with persistent Mullerian derivatives **(47)**.

Two female reared patients, who presented with primary amenorrhea and hypertension, had 17 alpha hydroxylase deficiency which is a very rare form of CAH; hypergonadotropic hypogonadism and mineralocorticoid hypertension are the two distinctive manifestations of the disorder **(20)**.

Twelve patients had XY gonadal dysgenesis. 46,XY gonadal dysgenesis could present in complete form; characterized by female external genitalia, well developed Müllerian ducts and gonads with no evidence of testicular differentiation, or partial form; characterized by partially developed mixture of Wolffian and Müllerian internal ducts with variable degrees of virilized external genitalia according to the preserved amount of testicular tissue **(35)**. Studying the genetic etiology of 46,XY gonadal dysgenesis has been a challenge; all together explain less than 40% of all cases **(9)**.

Three male reared patients had no trace of testes by laparoscopy confirming the diagnosis of testicular regression syndrome (TRS). Some males with TRS are born with normal male external genitalia and present with undescended testes or may even have unilateral or bilateral palpable testes that subsequently involute; the genetic etiology for this syndrome is still unknown **(35)**.

46, XX disorders of sex development included 3 subcategories; disorders of androgen excess in 66%, disorders of gonadal development in 29% and only 2 patients had other 46, XX DSD. Similar studies reported disorders of androgen exces as the commonest among 46,XX DSD **(27,56)**.

Congenital adrenal hyperplasia (CAH) was diagnosed mainly by clinical examination, imaging and biochemical testing with no genetic testing. Another study reached molecular genetic diagnosis in 21% of 46, XX DSD including patients with CAH **(27)**.

Twenty-four patients had CAH. It is considered the commonest cause of DSD which is reported in 1:15000 live births **(50)**. In our study CAH patients had a frequency of only 11% of total DSD patients. CAH can present with a wide range of manifestation, including variable degrees of virilization of female external genitalia **(15)**.

One male reared patient presenting with genital ambiguity had aromatase deficiency (AD), which is a rare autosomal recessive disorder; a total of 36 cases have been reported to date **(54)**, clinical presentation of patients varies depending on enzymatic activity, age and gender **(11)**. It leads to increase intrauterine androgen level with varying degrees of female external genital virilization **(54)**, and also possible maternal virilization during pregnancy **(10)**.

Eight male reared patients had testicular DSD, with no molecular diagnosis reached. 46,XX male syndrome is encountered infrequently in clinical practice; it is found approximately in 1 of 20,000-25,000 phenotypic males with 46,XX karyotype and typically present with almost normal male phenotype at birth **(59)**. Irrespective of being SRY positive or negative; 46,XX males would present with infertility due to the lack of AZF (a, b and c) regions which are involved in the regulation of normal spermatogenesis **(30)**.

Eight patients had histopathological diagnosis of Ovotesticular DSD (OT-DSD); half of them had sex chromosome OT-DSD. This is one of the rarest forms of DSD; it is a histopathological diagnosis of gonads harboring both ovarian and testicular tissue, patients can present with variable degrees of genital ambiguity as well as a mixture of Wolffian and Mullerian structures **(57)**. The most common karyotype of OT-DSD is 46,XX but other karyotypes were previously reported **(36,45,52)**.

Seventeen patients had DSD in association with other congenital anomalies representing 8%; 15 patients had 46, XY DSD and 2 patients had 46, XX DSD. Patients with ambiguous genitalia often have additional congenital anomalies **(22)**. Other congenital anomalies were reported to be associated with DSD in previous studies in 27% and 14% of patients **(16,21)**.

**Conclusions:**

The most frequent causes of DSD were TS 26%, KS 16%, CAH 12% and 5 alpha reductase deficiency 10%. This study enlarges the scope of cytogenetic abnormalities and monogenic mutations among DSD patients. Further studies, especially with the application of whole exome sequencing, are recommended to ensure the best management options for DSD patients.

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