Original Article

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Pathology Section

Disorders of Sexual Differentiation: A Clinicopathological and Histological Study

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ABSTRACT

Introduction: The term 'Disorders of Sexual Differentiation' (DSD) is defined as a congenital discrepancy between external genitalia, gonadal and chromosomal sex. The Chicago consensus based on the primary genetic defect is used for current nomenclature and classification. A child with DSD, if identified and treated early, may grow to become a well-adjusted, functional member of the society. Patients with DSD carry a low but definite risk of developing germ cell tumours which necessitates a histological analysis in all cases.

Aim: To describe the clinicopathological features of individuals with DSD and to classify the available cases according to the Chicago consensus which is based on karyotype and histopathology.

Materials and Methods: This retrospective descriptive study was done at a tertiary referral centre in South India, for a period of four months from May 2016 to August 2016. Case charts and histopathology slides of cases received in the Department of Pathology from January 2011 to May 2016 were included and reviewed. Cases where the histopathology slides/blocks were not retrievable/difficult to assess due to technical issues were excluded. Case charts and slides were reviewed retrospectively with a view to classify as per karyotypic or histological classification. The slides were reviewed by two Pathologists to help in the classification wherever possible. Statistical measures like mean and percentages were used to describe the results.

Results: A total of 22 cases of DSD were studied for which karyotype was available in 13 cases. Common clinical presentations were primary amenorrhoea, cryptorchidism and ambiguous genitalia. Age at presentation varied from nine months to 30 years. The initial diagnosis based on karyotype, clinical findings, hormonal assays and imaging were concordant with the final diagnosis in 11 cases. In two cases, histopathology was essential in arriving at a definitive diagnosis. Of the 13 cases with karyotype reports available, five were sex chromosome DSD, five were 46XY DSD and three were 46XX DSD. Gonadoblastoma was seen in three cases.

Conclusion: Histopathological examination together with karyotype plays a crucial role in proper categorisation of disorders of sexual differentiation. A multidisciplinary team approach for early diagnosis and management of DSD is essential for the psychosexual development of these individuals.

Keywords: Germ cell tumour, Gonadal dysgenesis, Karyotype

INTRODUCTION

Disorders of Sexual Differentiation (DSD) is defined as congenital conditions associated with atypical development of chromosomal, gonadal or anatomical sex [1]. The birth of a child with ambiguous genitalia heralds a long term pathophysiological aberration of gender identity, gender role and sexual orientation [2] as well as psychosocial issues for the individual and the family members. Hitherto used terminology like 'intersex', 'hermaphroditism' and 'pseudohermaphroditism' have been replaced by DSD [3]. The revised nomenclature as well as recommendations of treatment were laid down in a multidisciplinary meeting of medical and non medical experts at Chicago in 2006 (The Chicago consensus) [2].

The vast majority of DSD cases are 46XX DSD- congenital adrenal hyperplasia or 45XO/46XX DSD-mixed gonadal dysgenesis [3]. The incidence of DSD varies among different populations and is estimated to be 1:5500 worldwide [3]. Karyotype plays a central role in classification [2]; however clinical presentation, physical findings and specific investigations are equally important. The diagnosis and treatment require a multidisciplinary team, intervening at various stages to enable the individual to evolve into a well-adjusted member of the society [2].

Despite the large number of cases managed in clinical practice, there is a paucity of published literature on DSD data from the Indian subcontinent. This study presents a diagnostic histopathology perspective from a tertiary care referral and teaching centre of Karnataka.

Objective:

1. To study the clinicopathological features of individuals with DSD.
2. To classify the available cases according to the Chicago consensus which is based on karyotype and histopathology.
3. To correlate histopathology with clinical details including karytotype.
4. To highlight the importance of histopathology in picking up in-situ lesions early.

MATERIALS AND METHODS

A retrospective descriptive study was conducted at St. John's Medical College, Bangalore, Karnataka, India. All the cases received in the histopathology Department from January 2011 to May 2016 were reviewed between May 2016 and August 2016.

Inclusion criteria: All cases received in the Histopathology Department for tissue diagnosis during the mentioned time frame were included. Cases from other referral hospitals were also included.

Exclusion criteria: Cases where the histopathology slides/blocks were not retrievable/difficult to assess due to technical issues were excluded.

Study Procedure

A total of 22 cases received during the study period, were included. Case details of DSD cases received in the department were retrieved from the Paediatric Surgery Department archives. The clinical details including the age at presentation, sex of rearing and phenotype,

presenting complaints, physical findings, karyotype, hormonal profile, imaging and findings at genitoscopy/surgery were collected from the case records, if available. Several clinical details and results of preoperative investigations were lacking in five cases referred from elsewhere. The histopathology slides were reviewed by two Pathologists. The cases were classified as per the Chicago consensus [2], if karyotype was available. The rest of the cases were classified according to Aaronson's classification [1], based on histology of the gonad. This classification does not contradict Chicago consensus, but provides a better understanding based on the histology.

STATISTICAL ANALYSIS

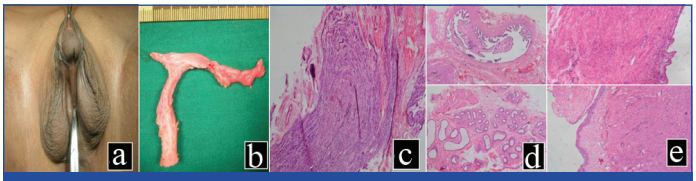
Statistical measures like mean and percentages were used to describe the clinical parameters using Statistical Package for the Social Sciences (SPSS) version 17.0.

RESULTS

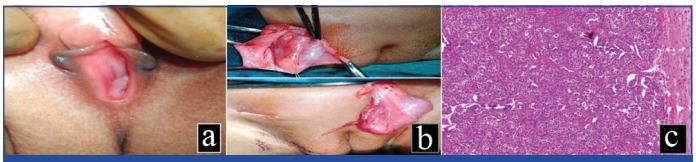
Twenty-two cases were included in the study. The mean age of presentation was 9.9 years. Total 12/22 (54.5%) of these cases were reared as females. Out of 13 cases (where karyotype reports were available), there were five sex chromosome DSD cases (45XO/46XY mixed gonadal dysgenesis), five 46XY DSD cases and three 46XX DSD cases [Table/Fig-1,2]. Of five 46XY DSD cases, the hormonal profile suggested Complete Androgen Insensitivity Syndrome (CAIS) and 17 beta-hydroxysteroid dehydrogenase deficiency in one each. There was one case of 46XX ovotesticular DSD [Table/Fig-3]. Gonadoblastoma was seen in 3/22 cases [Table/Fig-4]. Cases classified as per Chicago consensus are described in [Table/Fig-5]. In nine cases, where karyotype was not available, histological classification was done, among which testicular DSD was the most common [Table/Fig-6].

The provisional diagnoses based on clinical features, karyotype, imaging and hormonal assays were concordant with the final diagnosis in 11/13 cases. In two, a mistaken clinical diagnosis was clarified by the histopathology findings. The first case was referred as congenital adrenal hyperplasia (case 5 in [Table/Fig-5]) but histopathological examination of the gonads showed ovotesticular DSD. In the second case (case 12 of [Table/Fig-5]), the gonad of a suspected right inguinal heterogenous gonad akin to a polar ovotestis contained only testicular tissue. In this case, only the suspicious gonad with variable consistency was biopsied. The other gonad showed uniform texture with well-developed vas and vessels intraoperatively.

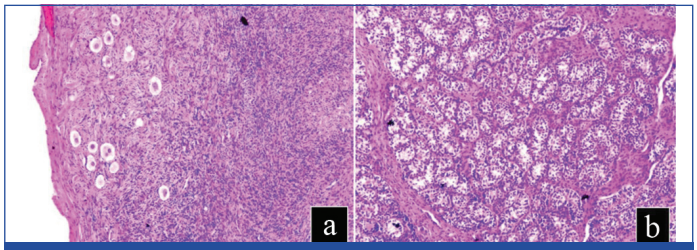
Several clinical details and results of preoperative investigations (hormonal profile, imaging, endoscopy) were lacking in five cases, including cases referred from elsewhere.



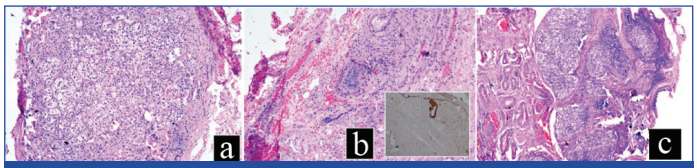
[Table/Fig-1]: a) 'Right descended gonad' and rugose scrotal folds b) Left hemiuterus intraoperatively c) Streak ovary (10X, H&E) d) Fallopian tube (upper panel, 4X, H&E), Tubular structures resembling epididymis (lower panel, 10X, H&E) e) myometrium (upper panel, 20X, H&E) e) Ectocervical tissue (lower panel, 10X, H&E) in a case of Mixed gonadal dysgenesis.



[Table/Fig-2]: a) Pseudovaginal pouch opening b) Gonads intraoperatively (right and left side) c) Testicular tissue (20X, H&E) in a case of 46XY DSD.



[Table/Fig-3]: a) Ovarian tissue with follicles (20X, H&E) b) Testicular tissue (20X, H&E) in a case of Ovotesticular DSD.



[Table/Fig-4]: a) Ectopic adrenal tissue (20X, H&E) b) Ovarian stroma with a focus of leydig cells highlighted by calretinin IHC (inset) (10X) c) Gonadoblastoma (20X, H&E).

Case number	Age, sex of rearing	Karyotype	Clinical details, imaging, endoscopy	Preoperative diagnosis	Histopathology	Final diagnosis
1.	13 years, Female	45XO/46XY	Ambiguous genitalia, Genitoscopy: Common urogenital channel. Laparoscopy: Right sided mullerian remnant, streak gonad Left intra abdominal gonad-? Testis	45XO/46XY Mixed gonadal dysgenesis	Left gonad- Testicular tissue. Right gonad not biopsied.	Sex chromosome DSD (45XO/46XY DSD)
2.	2 years, Male	45XO/46XY	Absent left scrotal gonad, descended right gonad Genitoscopy: Vagina, cervical impression: Laparoscopy: Right side- Vas and vessels Left-side: Intra abdominal streak gonad.	45XO/46XY Mixed gonadal dysgenesis	Left gonad- Streak gonad Ovarian stroma Mullerian remnant.	Sex chromosome DSD (45XO/46XY gonadal dysgenesis)
3.	5 years Male	46XX	Clinical details not available. Laparoscopy: Mullerian remnant.	Ovotesticular DSD	Left gonad- Ovarian tissue Mullerian remnant.	46XX DSD
4.	9 months Female	46XY	Clinical details not available. Intra abdominal gonads	46XY DSD	Bilateral gonadal biopsy- Immature testicular tissue, seminiferous tubules lined by sertoli cells.	46XY DSD
5.	6 years Female	46XX	Clinical details not available.	46XX DSD ? Congenital adrenal hyperplasia	Right gonad- ovarian tissue, many primordial follicles. Left gonad- Sertoli only seminiferous tubules.	46XX Ovotesticular DSD
6.	14 years Female	46XY	Pubertal virilisation, ambiguous genitalia. Primary amenorrhoea Bilateral inguinal palpable gonads Hormonal profile suggestive of (s/o) 17 Beta-hydroxysteroid dehyrogenase deficiency	46XY DSD	Bilateral gonads- Testicular and epididymal tissue.	46XY DSD (Disorders of androgen biosynthesis)
7.	18 years Female	46XY	Primary amenorrhoea. Ultrasound-Uterus and ovaries absent	46XY DSD	Bilateral gonads- ovarian stroma only, streak gonad. Focus of gonadoblastoma (left side)	46XY DSD, dysgenetic gonads Gonadoblastoma

8.	15 years Female	46XX	Primary amenorrhoea. Laparoscopy: Uterus, bilateral streak gonads	46XX DSD	Streak gonad, ovarian stroma with mesonephric remnants, Leydig cells.	46XX DSD, dysgenetic gonads
9.	7 years Male	45XO/46XY	Ambiguous genitalia, right impalpable gonad, left descended gonad Ultrasound- Mullerian remnant, right abdominal gonad	Mixed gonadal dysgenesis	Right gonad- Ovarian stroma, few seminiferous tubules lined by sertoli cells.	Sex chromosome DSD (45XO/46XY gonadal dysgenesis)
10.	19 years Female	46XY	Female phenotype, primary amenorrhoea Hormonal profile s/o androgen insensitivity	46XY DSD	Bilateral gonads- Testicular tissue.	46XY DSD (Complete androgen insensitivity syndrome)
11.	14 years Female	45XO/46XY	Primary amenorrhoea Laparoscopy: Hypoplastic uterus and fallopian tubes, bilateral streak gonads	Mixed gonadal dysgenesis	Bilateral gonads- Ovarian stroma, ectopic adrenal nest. Fallopian tubes unremarkable.	Sex chromosome DSD (45XO/46XY gonadal dysgenesis)
12.	14 months Female	46XY	Bilateral inguinal hernia, pseudovaginal pouch Right groin exploration- Gonad with variable consistency Left groin exploration- Uniform textured gonad with vas and vessels, not biopsied.	Ovotesticular DSD (Polar morphology)	Right gonad- Testicular tissue.	46XY testicular DSD
13.	30 years Male	45X (08), 46X, delY) (08)/47Xdel (Y), del (Y)	Impalpable undescended testes since birth Ultrasound- Lt. Mullerian remnant, adjacent streak gonad Atrophic right gonad at deep inguinal ring	Mixed gonadal dysgenesis/ ovotesticular DSD	Left gonad- Streak Right gonad- Testicular tissue	Sex chromosome DSD, dysgenetic

[Table/Fig-5]: Classification as per Chicago consensus where karyotype was available (original table) [2].

Case number	Age and phenotype	Clinical details	Histopathology	Histopathological classification
1.	17 years Female	Primary amenorrhoea. Mild clitoromegaly. Laparoscopy- Mullerian derivative, bilateral streak ovaries	Immature testicular tissue Focus of gonadoblastoma	Testicular DSD with gonadoblastoma
2.	Female	Not available	Immature testicular tissue with features of gonadoblastoma and an invasive germinoma	Testicular DSD with gonadoblastoma
3.	5 years Male	Persistent mullerian duct syndrome	Testicular tissue with few germ cells, right and left gonads	Testicular DSD
4.	9 years Male	Unilateral impalpable intra abdominal gonad	Dysgenetic left gonad	Dysgenetic DSD
5.	18 years Male	Bilateral impalpable undescended testis Laparoscopy-Uterus with bilateral? ovotestis	Testicular tissue, right and left gonads	Testicular DSD
6.	2 years Male	Not available	Right gonad-Testicular tissue Left gonad-Ovarian stroma.	Testicular dysgenetic DSD
7.	1 year Male	Bilateral undescended gonads	Right gonad-Testicular tissue with focal ovarian stroma c/w dysgenetic testis Left gonad- Testicular tissue	Testicular dysgenetic DSD
8.	9 years Male	Laparoscopy- Mullerian remnant	Right gonad-Testicular tissue Left gonad - ovarian stroma	Testicular dysgenetic DSD
9.	3 year Female	Bilateral intra abdominal gonads, near deep inguinal ring Genitoscopy- Common urogenital channel Hormonal profile s/o androgen insensitivity syndrome	No gonadal tissue seen	Not classified.

[Table/Fig-6]: Histological classification (n=9), where karyotype was not available [1].

DISCUSSION

Among 22 cases of DSD, the mean age at presentation was 9.9 years, much higher than the mean age of 27.4±38.4 months in another Indian study by Joshi RR et al., [5]. Only two cases in this study were diagnosed in infancy. Total 61.9% cases were identified after the age of five years, similar to the findings by Ammini AC et al., Rajendran R and Hariharan S [6,7]. A 12/22 cases were reared as females. However, the earlier quoted studies had more number of cases reared as males. A study from Saudi Arabia by Al-Jurayyan NAM had more cases reared as females [8]. Several cases reared as females presented at the pubertal age with primary amenorrhoea. A preferential male sex of rearing and late presentation is generally attributed to socio economic factors and stigma associated with DSD. There are no cases with congenital adrenal hyperplasia in this series, as they are generally diagnosed with investigations other than histopathology.

The DSD were traditionally classified into four groups: female pseudo hermaphroditism (46XX, ovarian gonads and variably virilised external genitalia), male pseudo hermaphroditism (46XY, testicular gonads, poorly virilised internal and/or external genitalia), gonadal dysgenesis (mosaic karyotypes, dysgenetic gonads) and true hermaphroditism (various karyotypes, combination of mature ovarian and testicular gonads) [9]. According to Chicago consensus and revised nomenclature [2], cases were classified as sex chromosome DSD (n=5, 38.4%), 46XY DSD, (n=5, 38.4%) and 46XX DSD (n=3, 23.07%). Rajendran R and Hariharan S have also reported 40% cases of 46XY DSD, similar to this study [7]. But in larger studies by Walia R et al., 46XX is the most common karyotype, the difference in this study could be due to the small sample size [10].

Of the five 46XY DSD cases, two had androgen insensitivity syndrome and one had 17 beta hydroxy steroid dehydrogenase deficiency (a defect of testosterone biosynthesis) [11]. All three were reared as females. There was only one case of ovotesticular DSD seen among the three cases of 46XX DSD's. Histopathology helps confirm a diagnosis of ovotesticular DSD as the karyotype can be one of a variety- 46XX, 46XY or mosaics [12]. Sex chromosome DSD constituted another major group, 45X/46XY mixed gonadal dysgenesis with ambiguous genitalia or asymmetric gonads was common. Once karyotype and the presence/absence of a Mullerian remnant are ascertained by imaging/endoscopy, gonadal histology helps in assigning a final diagnosis. The distinction is especially important between a dysgenetic state with dysgenetic gonads (streak) and an ovotesticular DSD with mature ovarian/testicular/ ovotesticular gonad. The distribution of ovarian and testicular

tissue within ovotestis varies, with one moiety likely to predominate and other assuming a polar or hilar distribution. Therefore, it is recommended that gonadal biopsies be taken from pole to pole with tissue chunk preferably from deep into the hilum of the gonad for confirmation [13].

Gonadoblastoma was detected in 3/22 cases (13.63%), of which two had immature testicular tissue and one developed in a streak gonad. In one, karyotype (46XY) was available. The prevalence of germ cell tumours in patients with gonadal dysgenesis is estimated at around 30% and in patients with undervirilisation syndromes at 5-10% [14,15]. Kathrins M and Kolon TF describes the overall tumour risk of dysgenetic DSD between 15% and 33%, similar to our findings [16]. The identification of in-situ precursor lesions in the gonad helps to decide between a strict wait and watch policy or a prophylactic gonadectomy to avert a subsequent invasive germ cell tumour [4]. The highest risk of neoplasia is found in Testis Specific Protein Y encoded (TSPY) positive gonadal dysgenesis as well as in the intraabdominal gonads in Partial Androgen Insensitivity Syndrome (PAIS). The lowest risk is associated with CAIS and ovotestis [2].

The workup of individuals with ambiguous genitalia is best done at the earliest to facilitate an informed decision on the gender of rearing and chart a calendar of medical and surgical management. The evaluation must include a detailed clinical examination of the child including the external genitalia. Thereafter, laboratory investigations including serum chemistry and hormonal profile, imaging and endoscopic assessment are individualised and tailored to unravel the final diagnosis in an algorithmic manner [11]. In fact, current prenatal diagnostic screening procedures include ultrasound, karyotyping from amniotic fluid, hormonal assays and molecular study of involved genes [3]. Future of DSD diagnosis also includes genetic study of various genes involved in gonadal development by molecular research methods [17,18]. This series underlines the importance of documentation of clinical and investigative details before submission of tissue (gonadal, genital ductal) for biopsy and further interpretation.

Limitation(s)

The paucity of clinical and investigative details in several of our patients as well as the non availability of representative gonadal/genital ductal tissue in some others limited the complete histopathological interpretation and classification of the entity.

CONCLUSION(S)

A karyotype is mandatory to categorise DSD. Hormonal profiling, imaging and endoscopy are important adjuncts in preoperative diagnosis. Histopathology of the gonads and internal genital ducts plays a confirmatory role. Early and accurate diagnosis of DSD facilitates optimal and multidisciplinary management of this difficult paediatric genitourological entity. It also facilitates surveillance of in-situ neoplastic lesions in the gonad prior to development of invasive germ cell tumours (low risk) or provides a basis for a prophylactic gonadectomy in high risk scenarios.

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