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Case Series

Pathology Section

Disorders of Sexual Development-Pathological Profile of 45 Cases at a Tertiary Care Centre

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ABSTRACT

Disorders of Sexual Development (DSD) are rare syndromes, which show congenital discordance between chromosomal, gonadal and phenotypic sex. A retrospective analysis was performed to analyse the histopathological profile and spectrum of a large number of cases of DSD received at a tertiary care centre. There were 45 cases of DSD encountered over a period of eight years, from January 2012 to December 2020. Detailed evaluation of each case with respect to demographic details, clinical features, imaging and pathology was done. All cases were classified as per Chicago Consensus Classification (2006) modified in 2010. The 46, XY DSD were the most common 26 (57.78%) cases, followed by sex chromosomal DSD 14 (31.1%) and 46, XX, DSD 5 (11.1%). Among 46, XY DSD, Complete Gonadal Dysgenesis (CGD) (Swyer syndrome) and Complete Androgen Insensitivity Syndrome (CAIS) had the highest number of cases, with 30.77% cases of each. Among 46XX, DSD, cases of ovotesticular DSD amounted to 80%. In sex chromosomal DSD, cases of Mixed Gonadal Dysgenesis (MGD) amounted to 78.57%. Out of 45 cases studied in this series, 20% cases showed neoplasms, of which 8.89% were malignancies. Nine out of 45 (20%) patients had neoplasms, out of which 5 (55.6%) had benign tumours while 4 (44.4%) had malignant tumours. Five patients had gonadoblastoma and three of these had co-existent dysgerminoma. Two patients had sertoli cell adenomas, one seminoma and one serous cystadenoma. Frequent clinical features noted were primary amenorrhoea seen in 25 (55.5%) cases and ambiguous genitalia seen in 18 (40%) cases, while the most common location of gonad was intra-abdominal in 30 (66.6%) cases. Streak gonads were seen in CGD, MGD and Turner's syndrome. Malignant germ cell tumours were seen in CGD and CAIS. Early diagnosis, good histopathology and follow-up are essential in the management of DSDs.

Keywords: Ambiguous genitalia, Gonadal dysgenesis, Gonadal neoplasms, Intersex, Sertoli cell adenomas

INTRODUCTION

The DSD are syndromes where there is congenital discordance between chromosomal, gonadal or phenotypic sex. Gonadal dysgenesis is defined as incomplete or defective formation of the gonads resulting from abnormal migration of the germ cells and/or their incorrect organisation in the foetal gonadal ridge. It is caused by structural or numerical anomalies of the sex chromosomes, or mutations in one of the genes involved in the formation of the urogenital ridge and sex determination of the gonad [1].

Genital ambiguity in newborns is a medical emergency with long term consequences and psychosocial effects on the patient as well as their families. Immediate gender assignment at birth is often necessary, requiring multidisciplinary team work as it depends on many factors like chromosomal karyotype, status of gonads as well as external genitalia [2,3].

Pathologists have an important role in identification of the gonads including Mullerian and Wolffian structures as well as identifying in-situ or invasive malignancies [4,5]. Malignant germ cell tumours have been found to arise in DSDs and in patients with gonadal dysgenesis who have a Y chromosome or Y chromosome material. They are at increased risk of developing germ cell tumours and an incidence of 10-30% has been reported in the literature [6-8]. The Chicago Consensus Classification (2006) updated in 2010, is currently used in diagnosing and classifying DSD, and it is primarily based on the karyotype [1].

CASE SERIES

This case series is based on the 45 gonadectomy specimens that were retrospectively reviewed. The specimens belonged to the period from January 2012 to December 2020 received in the Department of Pathology, at the tertiary care university hospital. The IEC approval was obtained vide letter number IEC(II)/OUT/1074/18.

Clinical data was obtained from the accompanying histopathological requisition forms and medical records of the hospital. The gonadal tissues were grossed at the time of receipt of specimens as per standard grossing protocols. Slides were prepared and stained with Haematoxylin and Eosin (H&E) for all cases. Special stains like Periodic acid Schiff, Masson trichrome as well as immunohistochemistry with Placental Alkaline Phosphate (PLAP), C-Kit, CD 30, inhibin, S-100, calretinin were done, wherever required for diagnosis.

Each of these 45 cases were re-evaluated and analysed according to the following criteria:

- Demographic details
- Clinical history and family history
- Physical examination including genital examination
- Karyotype profile (X and Y specific probe detection)
- Hormonal profile {Luteinising Hormone (LH), Follicle Stimulating Hormone (FSH), dihydrotestosterone, testosterone, androstenedione, 17 hydroxyprogesterone, Anti-mullerian Hormone (AMH)}
- The Ultrasonography (USG) and/or Magnetic Resonance Imaging (MRI) of abdomino-pelvic region for localisation of gonad and detection of Mullerian structures
- Routine laboratory investigations
- Pathology: gross, microscopy, immunohistochemistry

Features that suggested DSD were genital ambiguity, apparent male genitalia with micropenis, non palpable testis, isolated perineal hypospadias and apparent female genitalia with cliteromegaly, posterior labial fusion, and inguinal/labial mass.

Microscopic evaluation was done for identification of gonads as ovary/testis/ovotestis, identification of Mullerian or Wolffian structures, and detailed examination of the gonadal tissues for in-situ or invasive

malignancies. Identification of any cysts or associated pathology was also done.

Categorisation of DSD

Among these 45 cases, 26 cases (57.78 %) were 46, XY DSD, 14 cases (31.11%) were sex chromosome DSD and 5 cases (11.11%) were 46, XX DSD [Table/Fig-1]. Most of the cases (68.89%) were in the age group of 11-20 years. Ambiguous genitalia were seen in 18 cases (40%), male phenotype in 4 cases (8.89%) and female phenotype in 23 cases (51.11%) [Table/Fig-2]. Clinical features varied as per aetiology, primary amenorrhoea being the most frequent presenting feature in 25 cases (55.5%), followed by ambiguous genitalia in 18 cases (40%) [Table/Fig-3]. The location of the gonads in these patients was variable and included locations of intra-abdominal, inguinal, labio-scrotal, scrotal and inguinal, scrotal and intra-abdominal, inguinal and intra-abdominal. However, the most common location of both gonads was intra-abdominal in 30 cases (66.67%), followed by inguinal in eight cases (17.78%).

Disorders of sexual development		Number of cases (%)
46, XY DSD	Total cases	26 (57.78%)
	Complete gonadal dysgenesis (Swyer syndrome)	08 (30.77%)
	Complete androgen insensitivity syndrome	08 (30.77%)
	5 α- reductase type 2 deficiency	03 (11.54%)
	17 α- hydroxylase deficiency	02 (7.69%)
	17 β- hydroxysteroid oxidoreductase deficiency	02 (7.69%)
	Partial gonadal dysgenesis	02 (7.69%)
	Partial androgen insensitivity syndrome	01 (3.84%)
Sex chromosome DSD	Total cases	14 (31.11%)
	45,XO/46,XY DSD- Mixed gonadal dysgenesis	11 (78.57%)
	Turner syndrome and variants	02 (14.28%)
	45,XO/46,XY DSD- Ovotesticular DSD	01 (7.14%)
46, XX DSD	Total cases	05 (11.11%)
	Ovotesticular DSD	04 (80%)
	Congenital adrenal hyperplasia with 21 hydroxylase deficiency	01 (20%)

[Table/Fig-1]: Categorisation of cases based on Chicago Consensus Classification of Disorders of Sexual Development (modified in 2010) n=45.

Disorders of sexual development	Age at presentation (years)			Phenotype		
	1-10	11-20	21-30	Male	Female	Ambig-uous
46, XY DSD (N=26)						
Complete gonadal dysgenesis	-	5	3	-	7	1
Complete androgen insensitivity syndrome	-	7	1	-	7	1
5-alpha reductase deficiency	-	3	-	-	1	2
17-alpha hydroxylase deficiency	-	2	-	-	1	1
17-beta hydroxysteroid oxidoreductase deficiency	-	2	-	-	1	1
Partial gonadal dysgenesis	1	-	1	-	-	2
Partial androgen insensitivity syndrome	-	-	1	-	-	1
46, XX DSD (N=14)						
46, XX Ovotesticular DSD	1	3	-	2	-	2
Congenital adrenal hyperplasia	1	-	-	-	-	1
Sex chromosomal DSD (N=5)						
Mixed gonadal dysgenesis	3	8	-	2	4	5
Turner syndrome	-	2	-	-	2	-
45XO/46XY Ovotesticular DSD	1	-	-	-	-	1
Total (%)	7 (15.55%)	32 (71.11%)	6 (13.33%)	4 (8.89%)	23 (51.11%)	18 (40%)

[Table/Fig-2]: Age at presentation and phenotype (n=45).

Clinical features	Number of cases (%)
Primary amenorrhoea	25 (55.5%)
Ambiguous genitalia (phallus-like structure, short penis, cliteromegaly, labio-scrotal fusion)	18 (40%)
Blind vaginal pouch	20 (44.4%)
Hypospadias	09 (20%)
Poorly developed secondary sexual characteristics (breast development, pubic hair, axillary hair, facial hair)	21 (46.7%)
Inguinal swelling	9 (20%)
Hirsutism	3 (6.7%)
Family history of disorders of sexual development	6 (13.3%)
Nephrotic syndrome	1 (2.2%)

[Table/Fig-3]: Common clinical features.

46, XY DSD

Eight cases (30.77%) of 46, XY (CGD)/Swyer syndrome were encountered [Table/Fig-4]. These showed classical clinical features, bilateral streak gonads and well developed Mullerian structures (fallopian tubal structures). Hypoplastic uterus was identified on radiology of all cases, which was preserved and not sent for histopathological examination. Five out of eight cases showed gonadoblastoma (viable as well as burnt out) and dysgerminoma was seen in three of these cases.

Case No.	Radiology	Gross features		Microscopic features	
		Right gonadal structure	Left gonadal structure	Right gonad	Left gonad
1	Hypoplastic uterus Right gonad- solid neoplasm Left gonad- N.V.	4×3×1 cm	Linear tissue 3.5 cm	Gonadoblastoma dysgerminoma streak gonad	Burnt out gonadoblastoma streak gonad
2	Hypoplastic uterus bilateral small linear gonads	1.3×0.7 cm	1.7×1.3×1 cm	Gonadoblastoma streak gonad	Gonadoblastoma dysger-minoma Streak gonad
3	Hypoplastic uterus Both gonads-N.V	1.4×0.5×0.2 cm Tub. str. 3 cm	1.5×1×0.4 cm Tub. str. 5 cm	Viable and burnt out gonadoblastoma streak gonad, F.T.	Burnt out Gonadoblastoma streak gonad, F.T.
4	Hypoplastic uterus Both gonads-N.V.	2×1×0.2 cm	1.5×0.9×0.2 cm	Gonadoblastoma streak gonad	Streak gonad
5	Hypoplastic uterus Both gonads-N.V.	1.8×0.8×0.3 cm Tub. str. 3.5 cm	1.8×0.6×0.2 cm Tub. str. 4 cm	Streak gonad scanty ovarian stroma, F.T.	Streak gonad scanty ovarian stroma, F.T.
6	Undescended testis blind vaginal pouch	1.8×1.5×1 cm Tub. str. 2 cm	2×2×1 cm Tub. str. 3 cm	Streak gonad F.T.	Streak gonad F.T.
7	Hypoplastic uterus Both gonads-N.V.	3×2 cm Tub. str. 3 cm	4×2.3 cm Tub. str. 3 cm	Streak gonad F.T.	Streak gonad F.T.
8	Normal sized uterus bil. streak gonads	1.4×1×0.5 cm	2.5×1.5×1 cm	Gonadoblastoma streak gonad	Gonadoblastoma dysger-minoma streak gonad

[Table/Fig-4]: 46,XY DSD - Complete Gonadal Dysgenesis/Swyer syndrome (n=8). Tub. str.: Tubular structure; N.V.: Not visualised; F.T.: Fallopian tube; Bil: Bilateral

46, XY DSD - CAIS was also second most common DSD and comprised of eight cases (30.77%) [Table/Fig-5]. There were well formed testes along with epididymis and spermatic cord. Two of these cases, showed bilateral sertoli cell adenomas as well as multiple hyperplastic sertoli cell nodules which is a very unusual finding. One of the cases showed unilateral seminoma in the background of immature testis.

Case No.	Radiology	Gross features		Microscopic features	
		Right gonadal structure	Left gonadal structure	Right gonad	Left gonad
1	Uterus- Not visualised Both testis- deep inguinal ring	2.8×2×1.5 cm, testis Tub. str. 3 cm	2.5×2×1 cm, testis Tub. str. 3 cm	Immature testis* Wolffian duct derivative Vestigial Mullerian duct structures	Immature testis* Wolffian duct derivatives Vestigial Mullerian duct structures
2	Hypoplastic uterus Right gonad- N.V. Left gonad small sized	2×1.5×1 cm, testis Tub. str. 2 cm	1.5×1.4×0.4 cm, testis Tub. str. 2 cm	Seminoma Immature testis* Epididymis, Rete testis	Immature testis* Epididymis, Rete testis
3	N.A	5×5×1.5 cm, testis Tub. str. 2.5 cm	5.6×4×1.5 cm, testis Tub. str. 1 cm	Immature testis* Leydig cell micronodules Epididymis, Vas deferens	Immature testis* Leydig cell micronodules Epididymis , Vas deferens
4	Uterus- N.V. Blind vaginal pouch	3.5×2×1.5 cm, testis Tub. str. 6 cm	4×2.5×1.5 cm, testis Tub. str. 7 cm	Immature testis* Epididymis, Rete testis, Vas deferens	Immature testis* Epididymis, Rete testis, Vas deferens
5	Hypoplastic uterus Blind vaginal pouch Bil. gonads- intra-abdominal	4×2.6×1 cm, testis	4.5×2.5×1 cm, testis	Immature testis* Vas deferens	Immature testis* Leydig cell micronodules Vas deferens
6	Uterus- N.V. Blind vaginal pouch	4.5×2×1 cm, testis Tub. str. 3 cm	5.4×1.4×1.2 cm, testis Tub. str. 3 cm	Immature testis* Vas deferens	Immature testis* Vas deferens
7	Uterus- N.V. Bilateral gonads- inguinal regions	3.1×1.8×1 cm, testis Tub. str. 0.8 cm	4.4×2.6×2.3 cm, testis Tub. str. 1 cm	Sertoli cell adenoma Hyperplastic Sertoli cell nodules Vas deferens	Sertoli cell adenoma Hyperplastic Sertoli cell nodules Vas deferens; Benign cysts
8	Uterus- N.V. Blind vaginal pouch	5×2.5×2.5 cm, testis Tub. str. 2 cm	4.5×2.5×2 cm, testis Tub. str. 2 cm	Sertoli cell adenoma Leydig cell hyperplasia Vas deferens; Benign cysts	Sertoli cell adenoma Leydig cell hyperplasia Vas deferens; Benign cysts

[Table/Fig-5]: 46,XY DSD cases - Complete Androgen Insensitivity Syndrome (CAIS)/Testicular Feminising Syndrome (n=8).
*Seminiferous tubules lined mainly by sertoli cells and few germ cells, no spermatogenesis seen
N.A: Not available; Tub. str.: Tubular structure; N.V.: Not visualised

Three cases of 46, XY 5- alpha reductase type 2 deficiency were seen in this series [Table/Fig-6]. All three cases showed easily identifiable, bilateral testis with epididymis and vas deferens.

The remaining 46, XY DSD included seven cases which were grouped under 'other aetiologies' [Table/Fig-7]; two cases of 17-alpha hydroxylase deficiency, 17-beta hydroxysteroid oxidoreductase deficiency, partial gonadal dysgenesis each and a single case of partial androgen insensitivity syndrome. All showed well-formed testis on gross examination, and immature/ hypoplastic testis on histology.

Case No.	Radiology	Gross features		Microscopic features	
		Right gonadal structure	Left gonadal structure	Right gonad	Left gonad
1	N.A.	3×2×1 cm, testis Tub. str. 3 cm	2.5×2×1 cm, testis Tub. str. 3 cm	Immature testis* Epididymis Vas deferens	Immature testis* Epididymis Vas deferens
2	N.A.	4×2.5×2.5 cm, testis Tub. str. 4.5 cm	3.5×2.5×2 cm, testis Tub. str. 4 cm	Immature testis* Epididymis Rete testis	Immature testis* Epididymis Rete testis
3	Right gonad- inguinal region Hypoplastic left gonad- inguinal region; No Mullerian structures	4.5×3.2×2.1 cm, testis Tub. str. 4.5 cm	4×1×0.5 cm, testis Tub. str. 1.5 cm	Immature testis* Epididymis Vas deferens	Hypoplastic testis** Vas deferens

[Table/Fig-6]: 46, XY DSD, 5-Alpha Reductase Type 2 Deficiency (n=3).
* Seminiferous tubules lined mainly by Sertoli cells and few germ cells, no spermatogenesis seen
**3-4 seminiferous tubules lined by with few Sertoli and interstitium showing few Leydig cells
N.A: Not available; Tub. str.: Tubular structure

Case No.	Etiology	Radiology	Gross features		Microscopic features	
			Right gonadal structure	Left gonadal structure	Right gonad	Left gonad
1	17-Alpha Hydroxylase Deficiency	N.A	1.5 cm, testis	1.5 cm, testis	Immature testis*	Immature testis*
2		Blind vaginal pouch Bil. gonads- inguinal	4.5×3×2.5 cm, testis	4×3×2 cm, testis	Immature testis*	Immature testis*
3	17-beta hydroxysteroid oxidoreductase deficiency	N.A	3×1×0.5 cm, testis Tub. str. 1 cm	3.5×2×1 cm, testis Tub. str. 1 cm	Immature testis* Prominent Leydig cells, Vas deferens	Immature testis* Prominent Leydig cells, Vas deferens
4		Uterus- N.V. Bilateral gonads inguino-labial region	4.4×2.5×1.5 cm, testis Tub. str. 4 cm	3.2×1.5×1.5 cm, testis Tub. str. 3 cm	Immature testis* Epididymis, Vas deferens	Immature testis* Epididymis, Vas deferens
5	Partial Gonadal Dysgenesis	Uterus-well formed Bil. streak gonads-intra-abdominal	0.4×0.2 cm	0.8×0.1 cm	Hypoplastic testis**	Hypoplastic testis**
6		Hypoplastic uterus	2.5×1.5×1 cm, testis Tub. str. 2.5 cm	4×3.5×2 cm, testis Tub. str. 1.5 cm	Immature testis* Epididymis Vas deferens	Immature testis* Epididymis
7	Partial androgen insensitivity syndrome	Right gonad- hemiscrotum Left gonad- inguinal canal	3.5×3.2×1.5 cm, testis Tub. str.- 1.5 cm	3.5×2.5×1.5 cm, testis Tub. str.- 1.5 cm	Immature testis* Epididymis Rete testis Vas deferens	Immature testis* Epididymis Rete testis Vas deferens

[Table/Fig-7]: 46, XY DSD cases - other aetiologies (n=7).
*Seminiferous tubules lined mainly by sertoli cells and few germ cells, no spermatogenesis seen
**3-4 seminiferous tubules lined by with few sertoli and interstitium showing few Leydig cells
N.A: Not available; Tub. str.: Tubular structure; N.V.: Not visualised; Bil: Bilateral

Sex Chromosome DSD

Among MGDs, every case showed unilateral streak gonad and a contralateral dysgenetic testis/gonad. Seven cases showed fallopian tubal structure and one case showed additional Wolffian duct remnants. There were three cases with dysgenetic testes showing incomplete development of testis with four to five seminiferous

tubules lined mainly by sertoli cells and occasionally by germ cells [Table/Fig-8]. Hysterectomy was done in five cases and showed small uteri with proliferative endometrium.

Among sex chromosome DSD, there were two cases of Turner syndrome and its variant; and one case of ovotesticular DSD. Both the cases of Turner syndrome showed streak gonads with ovarian stroma and fallopian tubes; while one of these cases also showed Leydig cell clusters. A single case of ovotesticular DSD was also encountered which showed immature testis on the right side, ovarian stroma with primordial follicles on the left side, and structure of uterus [Table/Fig-9].

46, XX DSD

Four cases of 46, XX Ovotesticular DSD and a single case of congenital adrenal hyperplasia with 21 hydroxylase deficiency were also studied [Table/Fig-10]. Among the cases of 46, XX ovotesticular DSD, three cases showed unilateral ovotestis and ovarian tissue on the other side and all cases had a uterine structure. The ovarian tissue comprised of ovarian stroma arranged in whorls with cystic follicles. One of these cases was very unusual and also showed a serous cystadenoma of the ovary. The DSD with congenital adrenal hyperplasia showed bilateral ovarian stroma with primordial follicles, fallopian tubes and a structure of uterus.

DISCUSSION

All the 45 cases of DSD were encountered over a period of eight years. Among these 45 cases, 57.78 % were 46, XY DSD, 31.11% were sex chromosome DSD and 11.11% were 46, XX DSD.

Most of the cases were in the age group of 11-20 years. Clinical features varied as per aetiology; primary amenorrhoea being the usual presenting feature and female phenotype was the most common presentation, while ambiguous genitalia was the next frequent presentation. Bilateral gonads were intra-abdominal in most of the cases. Radiological investigations helped in

Sr. No.	Radiology	Gross features			Microscopic features		
		Right gonadal structure	Left gonadal structure	Other tissues	Right gonad	Left gonad	Other tissues
1	Hypoplastic uterus	Tub. str. 4 cm	1.5×1×0.2 cm	Uterus 1.7×1×0.5 cm	Streak gonad (Ovarian stroma)	Immature Testis*	Uterus- prol. endometrium Bil. F.T.
2	Hypoplastic uterus Scrotal sac empty	0.8×0.3×0.5 cm	1×0.5×0.5 cm	Uterus 1.5×1×0.5 cm, Tub. str. 3 cm	Streak gonad	Dysgenetic testis	Uterus-prol. endometrium Bil. F.T.
3	Hypoplastic uterus	Not removed	2×1×0.5 cm	Tub. str. 5×1 cm	-	Streak gonad	Uterus- prol. endometrium
4	Right gonad- External inguinal ring Left gonad(testis)- scrotal	5×2.7×1.7 cm	Not removed	Tub. str.- 2.5×1.8 cm	Streak gonad	-	Uterus-prol. endometrium Right F.T.
5	Hypoplastic uterus Right gonad- inguinal canal Left - pelvic	1.5×1×0.5 cm	Not removed	Not removed	Immature testis*	Not removed	Not removed
6	Complete agenesis of Mullerian structures (Uterus, Both ovaries- N.V.)	4×1×0.5 cm Tub. str. 1.5 cm	5×3×0.5 cm Tub. str.- 1.5 cm	-	Hypoplastic testis** Epididymis Vas deferens	Dysgenetic testis Epididymis Vas deferens	-
7	Bilateral gonads- intra-abdominal	0.5×0.5 cm	Tub. str.- 1.5×0.5 cm	Tub. str.- 1.5×0.5 cm	Immature testis*	Streak gonad	Uterus- Prol. endometrium Fallopian tube
8	Right gonad- intra-abdominal Left gonad- Normal testis in scrotum	3×1.5×1 cm	Not removed	-	Streak gonad	Not removed	-
9	N.A	Tub. str.- 6 cm	1×0.4×0.3 cm	-	Streak gonad	Dysgenetic testis	Fallopian tube
10	Hypoplastic uterus	3.5×1.5×0.5 cm	4×1×0.6 cm	Not removed	Wolffian duct structures	Streak gonad Wolffian duct structures	Bilateral fallopian tubes
11	Hypoplastic uterus	Tub. str. -2.5 cm	1.5×1×0.8 cm Tub. str. -2 cm	Not removed	Immature testis Streak gonad (Ovarian stroma)	Streak gonad	Left fallopian tube

[Table/Fig-8]: Sex Chromosome DSD - Mixed Gonadal Dysgenesis (n=11).
*Seminiferous tubules lined mainly by sertoli cells and few germ cells, no spermatogenesis seen
**3-4 seminiferous tubules lined by with few sertoli and interstitium showing few Leydig cells
N.A: Not available; Tub. str.: Tubular structure; N.V.: Not visualised; F.T.: Fallopian tube; Bil: Bilateral; Prol: Proliferative

Aetiology	Radiology	Gross features			Microscopic features		
		Right gonadal structure	Left gonadal structure	Other tissues	Right gonad	Left gonad	Other tissues
Turner syndrome and variants	Hypoplastic uterus Bilateral streak gonads	1.5×0.8×0.5 cm Tub. str.-2 cm	1×0.5×0.3 cm Tub. str.-1.5 cm	Not removed	Streak gonad Ovarian stroma F.T.	Streak gonad Ovarian stroma F.T.	Not removed
	Agenesis of uterus	2.5×0.5×0.3 cm Tub. str.-2.5 cm	3×0.5×0.3 cm Tub. str.-3.5 cm	-	Streak gonad Ovarian stroma Few Leydig cell clusters; F.T.	Streak gonad Ovarian stroma Few Leydig cell clusters; F.T.	-
45XO/46XY DSD- Ovo-testicular DSD	Inguinal canal: Right- ovotesticular like tissue Left- Ovary, testicular like tissue; uterus	0.2×0.1×0.1 cm	6×3×1.5 cm	Uterus 2.5×1.5× 0.5 cm	Immature testis*	Ovarian stroma with primordial follicles	Uterus- prol. endo F.T.

[Table/Fig-9]: Sex Chromosome DSD – Turner Syndrome and 45, XO/46,XY Ovotesticular DSD (n=3).
*Seminiferous tubules lined mainly by sertoli cells and few germ cells, no spermatogenesis seen
N.A: Not available; Tub. str.: Tubular structure; N.V.: Not visualised; F.T.: Fallopian tube; prol endo: Proliferative endometrium

Case No.	46 XX DSD	Radiology	Gross features			Microscopic features		
			Right gonadal structure	Left gonadal structure	Other tissues	Right gonad	Left gonad	Other tissues
1	Ovotesticular DSD	Hypoplastic uterus Both gonads- intra-abdominal	3.2×3×1 cm	2×1 cm Tub. str. 7 cm	Uterus 9×8.5×1 cm Breast: right-16×10×6.5 cm left-15×11×6.5 cm	Ovotestis	Ovarian stroma with cystic follicles Fallopian tube	Uterus- Prol. endo Gynaecomastia
2		Hypoplastic uterus Right gonad- Inguinal Left ovary- pelvic	0.3×0.2 cm	2.6×1.7×0.6 cm	Uterus 8×3.5×1.2 cm Tub. str. 6.5 cm	Ovotestis	Ovarian stroma with cystic follicles	Uterus- Secretory endometrium F.T
3		Right gonad- intra-abdominal Left gonad- Normal testis in scrotum	1×0.5×0.2 cm	Not removed	Linear tissue 4 cm	Ovarian stroma with cystic follicles Immature testis	Not removed	Uterus- Prol. endo.
4		Normal sized uterus Both gonads- intra-abdominal	Tub. str.- 6 cm	8×6×4.5 cm	Uterus 8.7×5.5×2.5 cm	Ovotestis	Serous cyst-adenomas- Ovary	Uterus- Prol. endo.
5	Congenital adrenal hyperplasia with 21, hydroxylase deficiency	Hypoplastic uterus Both ovaries in pelvis	4×1.5 cm Tub.str. -4 cm	4x1.5 cm Tub. str.-4 cm	Tub. str.-5×1 cm	Ovarian stroma with primordial follicles F.T.	Ovarian stroma with primordial follicles F.T.	Uterus- Prol. endo.

[Table/Fig-10]: 46, XX DSD cases- ovotesticular DSD and congenital adrenal hyperplasia (n=5).
Tub. str.: Tubular structure; F.T.: Fallopian tube; Prol. endo.: Proliferative endometrium

localisation, size of the gonads and identification of Mullerian or Wolffian structures. They were especially helpful in the identification of streak gonads.

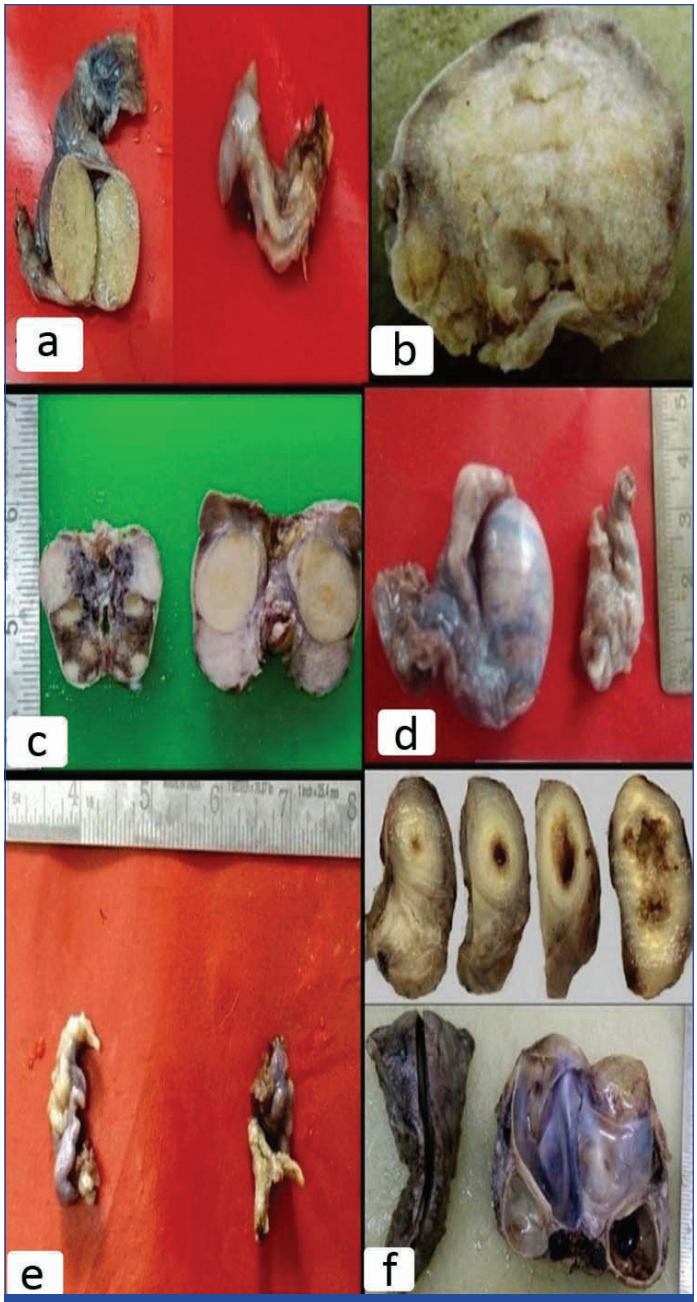
The gross examination of the gonadectomy specimens provided information about size of the gonad, presence of tumours and the presence of tubular structures, which on histopathological examination were Mullerian structures (uterus, fallopian tube) and Wolffian structures (rete testes, epididymis, vas deferens) [Table/Fig-11,12]. Well-formed testes were seen in 17 cases and were easily identified on gross examination. Heterogeneity of the gonadal tissue was seen and the entire gonad was needed to be sampled. The SOX9 for sertoli cells, FOXL2 for granulosa cells, OCT3/4, PLAP can be used in addition to the usual immunohistochemistry panel [9].

The most common DSD diagnosed in the present study was 45, XO/46, XY DSD-MGD (78.57%), followed by CGD (30.77%), and CAIS (30.77%). Congenital adrenal hyperplasia is the most prevalent DSD presenting with ambiguous genitalia at birth seen in 60-70% followed by MGD [10-12]. Most of these cases of DSD were unfortunately diagnosed at puberty or adulthood, compared to other studies available in literature, where DSD were mainly diagnosed at birth due to routine neonatal screening [2,10]. Poor governmental health policies, lack of routine neonatal screening, deliveries conducted at home or by paramedical staff and overall poor medical facilities could explain this delayed presentation at adulthood.

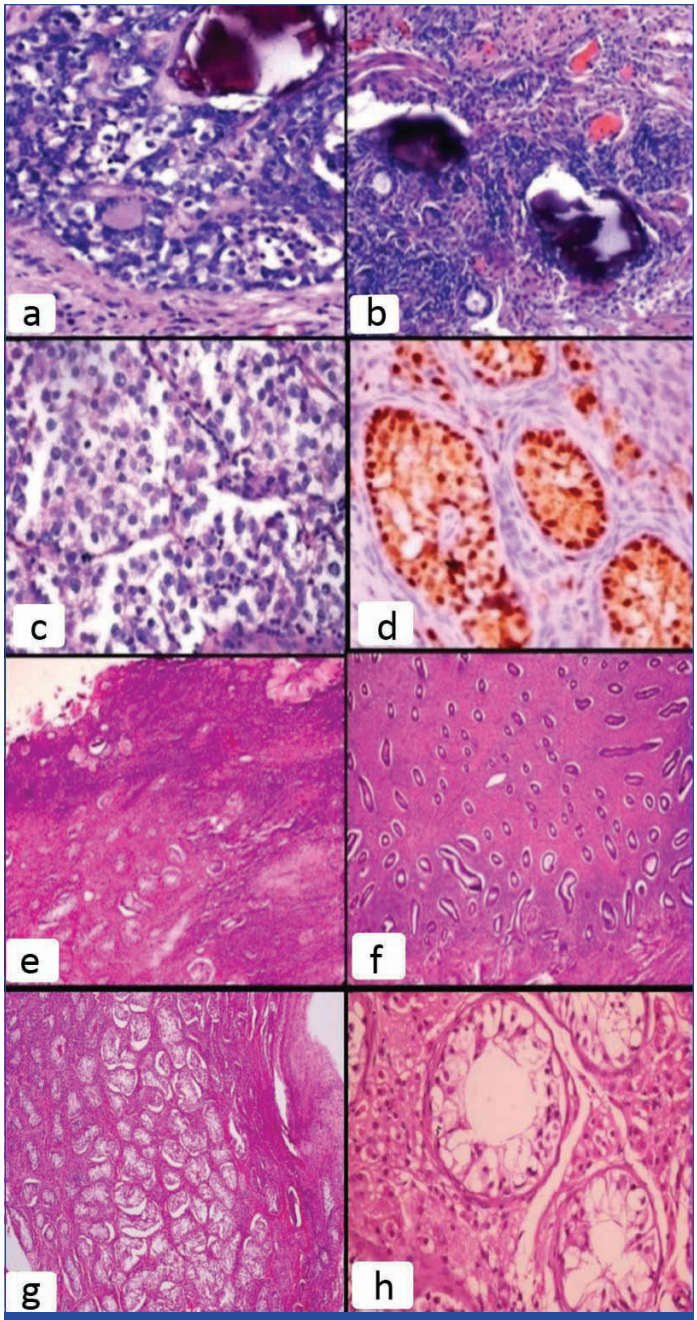
There were a number of spectacular cases in this study that are discussed here in brief. One of the cases of 46, XX ovotesticular DSD was very unusual and showed a serous cystadenoma of the ovary. This was a 21-year-old individual, with 46, XX karyotype, reared as a male, who presented with genital ambiguity, hirsutism, bilateral gynaecomastia, abdominal pain, cyclical bleeding through a single perineal opening below a 4 cm phallus-like structure and hypospadias. The right gonad was an ovotestis while the left gonad was replaced by an 8×6×4.5 cm, solid-cystic, ovarian neoplasm, composed of two locules with brown serous fluid. A uterus measuring 8.5×5.5×2.5 cm was also present. Histopathology of ovarian cyst confirmed a classic serous cystadenoma. Although the two pathologies have no specific association, their simultaneous occurrence deserves attention as reported by Zhao J et al., in their case report of serous borderline ovarian tumour in a case of 46, XY DSD [13].

Two cases of CAIS showing bilateral sertoli cell adenomas were also studied. One of these was an 18-year-old individual with a 46, XY karyotype, female phenotype with poorly developed secondary sexual characteristics, who presented with bilateral inguinal swellings and primary amenorrhoea. The clinical diagnosis was CAIS where no uterus was visualised and both the gonads showed characteristic sertoli cell adenomas and hyperplastic sertoli cell nodules [14]. The hyperplastic nodules were unencapsulated and composed of elongated immature sertoli cells arranged in well-formed tubules. No germ cells were seen admixed with the sertoli cells. Clusters of Leydig cells were seen in between the nodules. The sertoli cell adenomas were grossly encapsulated and golden-yellow, while microscopically composed of tubules lined by sertoli cells and separated by scanty fibrous tissue. Immunohistochemistry with calretinin strongly highlighted and confirmed sertoli cells and Leydig cells. These sertoli cell adenomas and hyperplastic nodules have been considered neoplastic or hamartomatous, interchangeably in literature [7,9]. The second case was a 16-year-old individual with 46 XY karyotype, apparent female genitalia, bilateral inguinal swellings, absent uterus, short vagina with development of breast and pubic hair. Both the inguinal gonads showed multiple sertoli cell adenomas as well as Leydig cell hyperplasia.

A familial case of CGD was also encountered in which all the four children presented with classical clinical features of Swyer syndrome. They had germ cell tumour in the backround of streak gonads. The eldest sibling had bilateral gonadoblastoma with dysgerminoma of the left gonad, the second sibling had bilateral gonadoblastoma with streak gonads, the third sibling had right sided gonadoblastoma and left sided streak gonad while the youngest sibling had bilateral streak gonads. The gonadoblastomas (GB) showed varying growth patterns and comprised of circumscribed nests of neoplastic germ cells with sex cord stromal cells surrounded by basement membrane deposits and superimposed by calcifications [9]. Most of the cases of GB in this study showed rounded basement membrane deposits and individual germ cells surrounded by a palisade of sex-cord cells. Dysgerminoma showed characteristic histomorphological features comprising of polyhedral cells with large nuclei,



[Table/Fig-11]: a) Mixed gonadal dysgenesis: Gonad (Immature testis) measuring 0.5×0.5 cm with attached tubular structure (uterus) measuring 1.5×0.5 cm.; b) Complete gonadal dysgenesis: Gonadal structure measuring 1.7×1.3×1 cm which showed predominantly dysgerminoma and focal areas of gonadoblastoma; c) Complete androgen insensitivity syndrome: Right gonadal structure measuring 3×1.8×1 cm and left gonadal structure measuring 4.4×2.6×2.3 cm, showing prominent yellowish- white nodules of sertoli cell adenoma (largest nodule) and sertoli cell hyperplasia; d) 5 alpha reductase deficiency: Right gonad (Immature testis) measuring 4.5×3.2×2.1 cm and left gonad (hypoplastic testis) measuring 4×1×0.5 cm; e) Mixed gonadal dysgenesis: Right gonad measuring 3.5×1.5×0.5 cm showing Wolffian duct structures only and left gonad measuring 4×1×0.6 cm showing streak gonad and Wolffian duct structures; f) 46 XX ovotesticular DSD: Well-formed uterus with cervix; Left ovary showing serous cystadenoma with 2 locules measuring 6×6×4 cm and 3×3×2 cm.



[Table/Fig-12]: a) Complete gonadal dysgenesis: Viable gonadoblastoma in streak gonad (H&E x400); b) Complete gonadal dysgenesis: Burnt out gonadoblastoma in streak gonad (H&E x400); c) Complete gonadal dysgenesis: Dysgerminoma (H&E x400); d) 46 XX ovotesticular DSD: Immunohistochemistry with Calretinin strongly highlighting sertoli cells and Leydig cells, stained deep brown; e) 46 XX ovotesticular DSD: Ovotestis- Ovarian stroma with corpus albicans and testis with immature seminiferous tubules. (H&E x100); f) 46 XX ovotesticular DSD: Uterus with proliferative endometrium (H&E x400); g) Complete androgen insensitivity syndrome: sertoli cell adenoma (H&E x100); h) 5 alpha-reductase deficiency: Hypoplastic testis showing seminiferous tubules lined by sertoli cell surrounded by Leydig cells (H&E x400).

prominent nucleoli and clear cytoplasm. Immunohistochemistry for PLAP and CD117 was done for confirmation of the diagnosis. A similar study had been reported by Eunice M et al., with one of the siblings having gonadoblastoma and the other two having streak gonads [15].

Malignant germ cell tumours were seen in CGD and CAIS [16] and gonadoblastomas were seen exclusively in CGD and were similar to studies in literature. Streak gonads were seen in CGD, MGD and Turner syndrome. Hence, streak gonads, though small and fibrotic need to be removed as they can harbour germ cell malignancies. sertoli cell adenomas as well as Leydig cell hyperplastic nodules were seen only in CAIS. Well-formed testes with epididymis and vas deferens were seen in 5-alpha reductase type two deficiency. CGD and MGD showed a strong family history of the disease.

We could not find many detailed pathology studies of DSD in literature despite the extensive search done, for comparative purposes; as most of the studies in literature are clinical studies. The studies which included histopathology were restricted to case reports and small series of cases of individual syndromes [15,17]. Hence, we have very few aetiological comparative studies and some with single entity, in this paper [2,12,15,17] [Table/Fig-13].

The DSDs require a multidisciplinary approach for diagnosis and treatment. Treatment includes gender assignment, surgical genital reconstruction, prophylactic gonadectomies, hormonal supplementation, psychosocial counselling and periodic follow-ups to rule out the development of neoplasms.

Disorders of sexual development		Present study (n=45)	Walia R. et al., (n=194) [2]	Eunice M et al., (n=3) [15]	Misgar RA et al., (n=73) [12]	Nonomura N et al., (n=65) [17]
46, XY DSD	Gonadal dysgenesis	10 (38.46%)	11 (10.8%)	3	1 (3.4%)	N.A.
	Androgen insensitivity syndrome	9 (34.61%)	32 (31.4%)	N.A.	7 (24.1%)	N.A.
	Androgen biosynthetic defect	4 (15.38)	26 (25.5%)	N.A.	2 (6.9%)	N.A.
	5-alpha reductase deficiency	3 (11.53%)	9 (8.8%)	N.A.	17 (58.6%)	N.A.
46, XX DSD	Ovotesticular	4 (80%)	8 (10.8%)	N.A.	1 (3.4%)	N.A.
	Congenital adrenal hyperplasia	1 (20%)	52 (70.3%)	N.A.	24 (82.7%)	N.A.
Sex chromosome DSD	Mixed gonadal dysgenesis	11(78.57%)	5 (71.4%)	N.A.	1 (6.7%0	65
	Ovotesticular	1 (7.14%)	1 (14.3%)	N.A.	N.A.	N.A.
	Turner syndrome and variants	02 (14.28%)		N.A.	07 (46.6%)	N.A.
Gonadal neoplasms		20%	N.A.	33.33%	N.A.	16.92%

[Table/Fig-13]: Comparison studies for aetiological classification of DSD [2,12,15,17].
*Clinical studies

CONCLUSION(S)

Role of histopathology is crucial to the diagnosis of DSD for identification of gonads, identification of Mullerian and/or Wollfian structures and identification of malignancies. An entire spectrum of morphologies was seen on the histopathology of gonads in DSD. Cases of 46 XY, DSD (57.78%) were the most frequently encountered in this tertiary centre. Among these, CGD (Swyer syndrome) and CAIS were the highest of all other aetiologies, amounting to 30.77% cases each. But the most common aetiological cause of DSD was MGD with 78.57% cases in contrast to the common causes of DSD as reported in literature. Out of 45 cases studied in this series, 20% cases showed neoplasms, of which 8.89% were malignancies, thus stressing over the importance of early diagnosis, good histopathology and follow-up of these cases. Streak gonads were seen in CGD, MGD and Turner's syndrome. Malignant germ cell tumours were seen in CGD and CAIS.

Even though DSD cases are not frequent in histopathological reporting, a systematic approach for histological gonadal evaluation is required. Molecular diagnostics can pave the way as a future diagnostic modality for DSD.

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