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Original Article

Prevalence, distribution, and risk markers for the development of gonadal germ cell tumors in patients with certain types of disorders of sexual differentiation with Y chromosome – A retrospective study

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Abstract

Purpose: To study the prevalence, subtypes, and risk markers for the development of gonadal germ cell tumors (GCT's) among disorders of sexual differentiation (DSD) patients with the Y chromosome.

Materials and Method: Design: A retrospective review of the patient's case records from 2010 to 2020 in Government Medical College, Thiruvananthapuram, India was studied. The study participants included 54 subjects with DSD containing the Y chromosome. Demographic data, external masculinization scoring, associated congenital anomalies, karyotyping, intraoperative findings such as gonadal location and internal genital ducts, histopathology of the resected gonads, and its immunohistochemistry were collected. The prevalence of gonadal GCT's was estimated from paraffin-embedded gonadectomy samples (S = 82).

Results: The median age of occurrence of gonadal GCT's was 18 years. The prevalence of malignant gonadal GCT's was highest among the PAIS group (19.2%) followed by gonadal dysgenesis (15.8% each in MGD and CGD) and least among CAIS (7.7%) ($p < 0.01$). The most common type of malignant gonadal GCT's in the descending order of frequency was dysgerminoma, seminoma, mixed GCT, and yolk sac tumor. Multivariance logistic analysis showed post-puberty and the presence of congenital anomalies were associated with the occurrence of gonadal GCT's ($P < 0.01$).

Conclusion: The overall prevalence of gonadal GCT's (malignant and premalignant) among DSD with Y chromosomes is nearly 25%. Dysgerminoma is the most common malignant gonadal GCT's. Age at or above 18 years and the presence of congenital anomalies like renal agenesis, retroperitoneal vascular defects, and congenital diaphragmatic hernia were independent risk markers for the development of gonadal GCT's.

Keywords:
Congenital anomalies, DSD, Gonadal GCT's, Y chromosome

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Introduction

Gonadal germ cell tumors (GCTs) are neoplasms that can arise from dysgenetic gonads. GCT's include dysgerminomas, seminomas, yolk sac tumors, teratomas, choriocarcinomas, and premalignant lesions like gonadoblastomas.[1,2] The prevalence of GCT's among patients with disorders of sexual differentiation (DSD) varies.[3] Presence of Y chromosome or Y derived sequences in a dysgenetic gonad makes it vulnerable to the development of GCT's. DSD patients with the Y chromosome, like 46, XY complete and partial gonadal dysgenesis (CGD and PGD), androgen insensitivity syndrome- complete or partial (CAIS or PAIS), and 45, X/46, XY gonadal dysgenesis or mixed gonadal dysgenesis (MGD) have an increased risk of GCT's compared to the general population.[4] Studies claiming the statistics about the prevalence of GCT's in DSD are very few. This study aimed to find the prevalence, subtype, and to learn the risk markers for the development of GCT'S among DSD patients with the Y chromosome.

Materials and Method

The study was conducted in a Government Medical College, Thiruvananthapuram, Kerala after gathering approval from the ethics committee of the institute. We defined DSD subjects with Y chromosomes as following:

I, Cases with disorders of gonadal development which includes 46 XY gonadal dysgenesis [(CGD also called Swyer syndrome and/or PGD) and MGD].

II, Cases with disorders of androgen action (CAIS or PAIS).

Details of the above-defined DSD cases that had undergone gonadectomy from January 2010 to March 2020 were collected from the records library. Clinical data noted included demographic profile, sex of rearing, presentation, phenotype, description of the external genitalia, external masculinization score (EMS), other associated congenital anomalies, hormonal profile, karyotyping, location of gonads, and the type of internal ducts based on the laparoscopic or laparotomy documentation, histopathology of the resected gonads (testis, ovary, dysgenetic testis, streak, a combination of these or tumorous), type of germ cell tumor and immunohistochemistry of gonadectomy specimen. The indications for surgeries done among the CGD and MGD were considered at the time of diagnosis considering the possibility of malignant transformation after counseling and informed consent. CAIS subjects underwent gonadectomy after completing puberty and PAIS subjects with undescended testis with symptoms or suspicion of malignancy on routine examinations.

Diagnosis of 46 XY DSD subtypes was made based on the following features;

I. CGD was diagnosed, if one had a female phenotype, with a complete absence of testicular tissue (streak) with the presence of normal or rudimentary Mullerian structures and a 46 XY karyotyping.
II. MGD was diagnosed based on genital phenotype, gonadal phenotype, and mosaic karyotype. Genital phenotype ranged from female external genitalia or mild clitoromegaly through all the stages of ambiguous genitalia to hypospadias or a normal penis. Gonadal phenotype included streak gonads through dysgenetic testis to the normal testis. Karyotype included 45X/46XY or 45 X/47XXY or 45X/47 XYY or 45X/46XY/47XYY. 30 cell karyotyping was done in all cases. In conditions where 30 cell karyotyping didn't show Y chromosome and clinical suspicion was high, 100 cell karyotyping was done.
III. Androgen biosynthetic defect (ABD) included 5 alpha-reductase (5 and 17 β hydroxysteroid dehydrogenase (17 βHSD) deficiency. They were diagnosed based on the undervirilized genitals, presence of testis as gonad (scrotal or non-scrotal), 46 XY karyotype, and biochemical ratios of hormones after hCG stimulation test. The ratio of testosterone: dihydrotestosterone >10 and testosterone to androsteinedione <0.8 is suggestive 5of respectively.
IV. AIS: Androgen receptor mutation analysis was not available in the center and diagnosis of CAIS was based on female phenotype with 46 XY karyotyping with gonads being testis and positive androgen insensitivity test (AIT). PAIS was defined as incomplete masculinized with or without descended testis with 46 XY karyotype and positive AIT (subjects diagnosed AIS were in puberty as evident from hormonal data). Gonads in the scrotum were considered as testis. Inguinal gonads or abdominal gonads were considered testis based on the biopsy report. Scrotal testis was considered normal if the patient had a normal clinical examination finding and normal USG- inguinoscrotal reports pre-operatively.

hCG (human chorionic gonadotrophin) stimulation test: Baseline blood samples were collected in fasting for total testosterone. hCG was administered deep intra-muscular for three consecutive days in a dose based on the age of the patient (age <1 year 500 IU per day hCG, age 1-10 years 1000 IU per day, and age >10 years 1500 IU per day). Blood samples were collected 24 hours after the third dose for total testosterone, dihydrotestosterone, and androstenedione. The ratios of hormones were calculated.

Key Message

The prevalence of gonadal germ cell tumors among the DSD with the Y chromosomes is nearly 25%.

- *Adulthood and associated congenital anomalies involving the renal and vascular systems are a risk marker for the development of gonadal tumors.*

AIT [stanozolol sex hormone-binding globulin (SHBG) test]: This test was done to differentiate ABD and AIS. Oral stanozolol (0.2 mg/kg/day) was given for three consecutive days. Blood samples for SHBG were obtained as baselines and on days 5, 6, 7, and 8. The lowest serum SHBG measured on days 5 to 8 represented the nadir and was considered the largest response to stanozolol. The ratio of nadir serum SHBG to baseline serum SHBG <63.4% is considered normal. The nadir serum SHBG between 73% to 89% and 93% to 97% was considered positive for the diagnosis of PAIS and CAIS respectively.[5]

EMS: This is an objective score to measure the degree of virilization of genitals and takes into account features such as the presence or absence of scrotal fusion, micropenis, and location of each gonad and urethral meatus.[6]

Gonadectomy specimens and histopathology: All gonadal specimens were tissue fixed with 10% formalin for 24 hours followed by paraffin wax embedding and preparation of slides of 4-micrometer thickness. Hematoxylin- Eosin staining of the samples was done and classified as normal testis, dysgenetic testis, ovary, streak, a combination of these, or gonadal GCT (premalignant and malignant neoplasms). Premalignant, also called in-situ neoplastic lesions included carcinoma-in-situ (CIS) or gonadoblastoma or octamer binding transcription factor (Oct) 4 positivity for basal lamina cells of testicular tubules.[3,7] CIS diagnosis was made according to WHO criteria.[8] Malignant GCT included dysgerminoma, seminoma, yolk sac tumor, teratoma, or mixed GCT. All specimens were subjected to immunohistochemistry (IHC) staining. IHC staining used to stain GCT included Oct 3/4, placental alkaline phosphatase (PLAP), alpha feta-protein (AFP), CD-117 (c Kit), and inhibin.[9] Appropriate positive and negative controls for each antibody were analyzed.

Statistics

The prevalence of GCT was calculated based on the paraffin-embedded gonadal samples (S = 82). In each subgroup, the prevalence was calculated separately. A comparison between categorical variables was performed by using the Fischer exact test. Multivariance logistic analysis was used to find the association between the risk markers and the occurrence of gonadal GCT. *P* value <0.05 was considered significant.

Results

The median age of all subjects (*n* = 54) was 17.5 years. The ages of the study subjects ranged from 2 to 34 years. Out of the 54 subjects with 46 XY DSD; 10 cases were MGD (18.5%), 10 cases were CGD (18.5%) and 34 cases were AIS (63%). The most common initial presentation were pain and swelling over groin (*n* = 21, 39%) followed by delayed puberty (*n* = 15, 28%), ambiguity in genitals (*n* = 7, 13%), primary infertility (*n* = 5, 9%), virilisation (*n* = 3, 5.5%), short stature (*n* = 2; 4%) and haemoptysis (*n* = 1, 1.8%). Gonads were localized either in the abdomen, inguinal or scrotal regions. In one case of 46 XY CGD, we couldn't localize the gonad either radiologically or on laparotomy and was considered non-localized or vanishing. 82 gonadal tissue samples were available from 54 cases (80 gonadectomy and 2 biopsies). Bilateral gonadectomy was done in all cases of MGD, CGD, and CAIS. Gonadectomy was done at the time of diagnosis in MGD and CGD. CAIS subjects underwent the gonadectomy after the completion of puberty. PAIS subjects underwent unilateral gonadectomy either for suspected torsion testis which couldn't be salvaged or any suspicion of malignancy detected on clinical examination or ultrasound/CT imaging. Two cases of PAIS were having testis located in the abdomen and were removed as they were having a suspicion of malignancy on CT abdomen imaging at the time of presentation. None of the scrotal testes among the PAIS subjects had features of malignancy clinically or radiologically. No cases of ABD with gonadectomy reports were available. The number of gonadal GCT was 12 (premalignant 5 and malignant GCT 7) among the study subjects. The median age of occurrence of gonadal GCT was 18 years. One adult MGD patient presenting with hemoptysis was found to have lung secondaries due to dysgerminoma arising from a dysgenetic gonad Table 1. Subjects with malignant GCT's were followed up in the oncology department. The prevalence of gonadal tumors (overall and malignant) is summarized in

Table 1: The characteristics of study subjects DSD with Y chromosome (*n*=54)

Age of presentation (years)	Sex assigned	Initial presentation	karyotype	Gonad-al Location Right	Gonad-al Location Left	EMS	Gonadal Tissue Type Right (histology)	Gonadal Tissue Type Left (histology)	Diagnosis	Surgery done	Congenital anomalies	Oct 3/4	PALP	AFP	CD-117	Inhibin
2	F	Short stature	45X/46XY	AB	AB	1	Streak	DT	MGD	Bilateral gonadectomy	-	—	-	-	-	-
3	F	Short stature	45X/46XY	AB	AB	1	Streak	DT	MGD	Bilateral gonadectomy	URA	- -	-	-	-	-
3	F	ambiguous genital	45X/46XY	AB	AB	4	Streak	DT	MGD	Bilateral gonadectomy	-	- -	-	-	-	-
4	F	ambiguous genital	45X/46XY	AB	AB	4	DT	Streak	MGD	Bilateral gonadectomy	-	- -	-	-	-	-
4	F	ambiguous genital	45X/46XY	AB	AB	4	DT	Streak	MGD	Bilateral gonadectomy	-	- -	-	-	-	-
15	F	clitoromegaly	45X/46XY	AB	AB	5	DT	Streak	MGD	Bilateral gonadectomy	-	- -	-	-	-	-
16	F	clitoromegaly	45X/46XY	AB	AB	4	Gondoblastoma from DT	Streak	MGD	Bilateral gonadectomy	URA	- +	+	-	-	-
16	F	clitoromegaly	45X/46XY	AB	AB	5	Mixed GCT (Yolk sac and dysgermi-noma)	Streak	MGD	Bilateral gonadectomy RLND	URA	+ +	+	+	+	-
18	M	Delayed puberty	45X/46XY	AB	I	9.5	Dysgerminoma from DT	Testis	MGD	Right gonadectomy Left Orchidopexy with biopsy	UAR	+ +	+	-	-	-
34	M	hemoptysis	45X/46XY	AB	I	9.5	Dysgerminoa From DT	Testis	MGD	Right gonadectomy with RLND Left Orchidopexy with biopsy	-	+ +	+	-	-	-
15	F	Primary Ammenoherrea	46XY	AB	AB	1	Streak	Streak	CGD	Bilateral gonadectomy	-	- -	-	-	-	-
16	F	Primary Ammenoherrea	46XY	NL	NL	0	Streak	Streak	CGD	Bilateral gonadectomy	-	- -	-	-	-	-
16	F	Primary Ammenoherea	46XY	AB	AB	1	Streak	Streak	CGD	Bilateral gonadectomy	-	- -	-	-	-	-
16	F	Primary Ammenoherea	46XY	AB	AB	1	Streak	Streak	CGD	Bilateral gonadectomy	-	- -	-	-	-	-
16	F	Primary Ammenoherea	46XY	AB	AB	1	Dysgerm-inoma	Streak	CGD	Bilateral gonadectomy with RLND	CDH	+ +	+	-	-	-
18	F	Primary Ammenoherea	46XY	AB	AB	1	Gonadob-lastoma	Streak	CGD	Bilateral gonadectomy	-	- +	+	-	-	+

Contd...

Table 1: Contd...

Age of presentation (years)	Sex assigned	Initial presentation	karyotype	Gonad-al Location Right	Gonad-al Location Left	EMS	Gonadal Tissue Type Right (histology)	Gonadal Tissue Type Left (histology)	Diagnosis	Surgery done	Congenital anomalies	Oct 3/4	PALP	AFP	CD-117	Inhibin
18	F	Primary Ammenoherea	46XY	AB	AB	1	Streak	Dysger minoma	CGD	Bilateral gonadectomy with RLND	-	- -	+	-	-	-
18	F	Primary Ammenoherea	46XY	AB	AB	1	Mixed germ cell tumor (Yolk sac and seminoma)	Streak	CGD	Bilateral gonadectomy with RLND	LIVC	+ +	+	+	+	-
18	F	Primary Ammenoherea	46XY	AB	AB	1	Streak	Streak	CGD	Bilateral gonadectomy	-	- -	-	-	-	-
18	F	Primary Ammenoherea	46XY	AB	AB	1	Streak	Streak	CGD	Bilateral gonadectomy	-	-	-	-	-	-
18	F	Primary Ammenoherea	46XY	AB	I	1.5	Testis	Testis	CAIS	Bilateral gonadectomy	-	—	-	-	-	-
18	F	Primary Ammenoherea	46XY	I	I	2	CIS	Testis	CAIS	Bilateral gonadectomy	-	- +	+	-	-	-
20	F	Primary Ammenoherea	46XY	I	I	2	Testis	Testis	CAIS	Bilateral gonadectomy	-	-	-	-	-	-
20	F	Primary Ammenoherea	46XY	I	I	2	CIS	Testis	CAIS	Bilateral gonadectomy	-	- +	+	-	-	-
22	F	Primary infertility	46XY	AB	AB	1	Testis	Testis	CAIS	Bilateral gonadectomy	-	-	-	-	-	-
24	F	Primary infertility	46XY	I	AB	1.5	Testis	Testis	CAIS	Bilateral gonadectomy	-	-	-	-	-	-
28	F	Primary infertility	46XY	I	I	2	Testis	CIS	CAIS	Bilateral gonadectomy	-	- +	+	-	-	-
32	F	Primary infertility	46XY	I	I	2	Testis	Dysge-rminoma	CAIS	Bilateral gonadectomy RLND	UAR	+ +	+	-	-	-
12	M	pain in left groin	46XY	S	I	7.5	Testis	Infarcted Testis	PAIS	Left gonadectomy		- -	-	-	-	-
12	M	pain in left groin	46XY	S	I	7.5	Testis	Infarcted Testis	PAIS	Left gonadectomy	-	- -	-	-	-	-
14	M	pain in left groin	46XY	S	I	10.5	Testis	Infarcted Testis	PAIS	Left gonadectomy	-	- -	-	-	-	-
14	M	pain in left groin	46XY	S	I	11	Testis	Testis	PAIS	Left gonadectomy	-	- -	-	-	-	-

Contd...

Table 1: Contd...

Age of presentation (years)	Sex assigned	Initial presentation	karyotype	Gonad-al Location Right	Gonad-al Location Left	EMS	Gonadal Tissue Type Right (histology)	Gonadal Tissue Type Left (histology)	Diagnosis	Surgery done	Congenital anomalies	Oct 3/4	PALP	AFP	CD-117	Inhibin
15	M	pain and swelling right groin	46XY	I	S	10.5	Mixed germ cell tumor (yolk sac tumor and seminoma)	Testis	PAIS	Right Gonadectomy with RLND	URA	+ +	+	+	+	-
15	M	pain in left groin	46XY	S	I	11	Testis	Testis	PAIS	Left gonadectomy		- -	-	-	-	-
16	M	pain in right groin	46XY	I	S	11	Testis	Testis	PAIS	Right gonadectomy	-	- -	-	-	-	-
16	M	pain and swelling right over groin	46XY	I	S	10.5	hemorrhagic and infarcted testis	Testis	PAIS	Right Gonadectomy	-	- -	-	-	-	-
16	M	pain and swelling right over groin	46XY	I	S	10.5	hemorrhagic and infarcted testis	Testis	PAIS	Right Gonadectomy	-	- -	-	-	-	-
16	M	pain and swelling left over groin	46XY	S	I	10.5	Testis	Yolk sac tumor	PAIS	Left Gonadectomy RLND	-	- -	+	+	+	-
16	M	pain and swelling left over groin	46XY	S	I	11	Testis	hemorrhagic and infarcted testis	PAIS	Right Gonadectomy	-	- -	-	-	-	-
17	M	pain in left groin	46XY	S	I	10.5	Testis	Testis	PAIS	Left Gonadectomy	-	- -	-	-	-	-
17	M	pain in left groin	46XY	S	I	11	Testis	Testis	PAIS	Left Gonadectomy	-	- -	-	-	-	-
17	M	pain in left groin	46XY	S	I	11	Testis	Testis	PAIS	Left Gonadectomy	-	- -	-	-	-	-
18	M	pain in left groin	46XY	S	I	11	Testis	Testis	PAIS	Left Gonadectomy	-	- -	-	-	-	-
18	M	pain and swelling left groin	46XY	S	I	10.5	Testis	hemorrhagic and infarcted testis	PAIS	Left Gonadectomy	-	- -	-	-	-	-
18	M	pain and swelling right over groin	46XY	I	S	10.5	Seminoma	Testis	PAIS	Right Gonadectomy with RLND	LIVC	+ +	+	-	-	-
18	M	pain and swelling right over groin	46XY	I	S	10.5	hemorrhagic and infarcted testis	Testis	PAIS	Right Gonadectomy	-	- -	-	-	-	-

Contd...

Table 1: Contd...

Age of presentation (years)	Sex assigned	Initial presentation	karyotype	Gonad-al Location Right	Gonad-al Location Left	EMS	Gonadal Tissue Type Right (histology)	Gonadal Tissue Type Left (histology)	Diagnosis	Surgery done	Congenital anomalies	Oct 3/4	PALP	AFP	CD-117	Inhibin
18	M	pain and swelling right over groin	46XY	I	S	11	Testis	Testis	PAIS	Right Gonadectomy	-	- -	-	-	-	-
18	M	ambiguous genital	46XY	I	S	11	Testis	Testis	PAIS	Right Gonadectomy	-	- -	-	-		-
20	M	ambiguous genital	46XY	I	S	11	Testis	Testis	PAIS	Right Gonadectomy	-	- -	-	-	-	-
20	M	pain and swelling left over groin	46XY	S	I	10.5	Testis	Semino-ma	PAIS	Left Gonadectomy with RLND	-	+ +	+	-	-	-
20	M	ambiguous genital	46XY	S	AB	10	Testis	DT	PAIS	Left gonadectomy	-	-	-	-	-	–
20	M	pain and swelling right over groin	46XY	S	I	10.5	Testis	Seminoma	PAIS	Left Gonadectomy RLND	-	+ +	+	-	-	-
20	M	ambiguous genital	46XY	S	AB	10	Testis	DT	PAIS	Left Gonadectomy	-	-	-	-	-	-
28	M	Primary infertility	46XY	S	I	11	Testis	Testis	PAIS	Left goandetomy	-	-	-	-	-	-

AB=Abdominal, I=Inguinal, NL=Not Localised, S=Scrotal, DT=Dysgenetic testis, LIVC=Left-sided IVC, CDH=Congenital diaphragmatic hernia, URA=Unilateral renal agenesis, RLND=Retro-peritoneal lymph node dissection, PLAP=Placental Alkaline Phosphatase, Oct 3/4=Octamer binding transcript 3 and 4 and AFP=Alpha-fetoprotein

Table 2. The distribution of gonadal GCT's is shown in Table 3. Congenital anomalies like renal agenesis, left-sided IVC, and CDH were seen among the study subject.

Showing the characteristics of study subjects DSD with the Y chromosome. (n = 54) Table 1.

The overall prevalence of gonadal GCT among DSD with the Y chromosome is 20.7%. The prevalence of gonadal GCT among MGD, CGD, CAIS, and PAIS is 20%, 20%, 25%, and 19.2% respectively Table 2.

The distribution of malignant gonadal tumors in the descending order of occurrence in the subjects were dysgerminoma (n = 5; 29.4%), seminoma (n = 3, 17.6%), mixed GCT (n = 3, 17.6%) and yolk sac tumor (n = 1, 5.8%) Table 3.

The multivariance logistic regression analysis showed that age at or above 18 years [OR 7.34 (CI 3.42-15.76) $P < 0.01$] and presence of congenital anomalies like renal agenesis, congenital diaphragmatic hernia, and retroperitoneal vascular defects [OR -3.09 (CI 1.69-5.65), $P < 0.01$] are independently associated with the occurrence of gonadal GCT's among the study subjects Table 4.

Discussion

The overall prevalence of GCT's among DSD patients with the Y chromosome ranged from 20-25% in our series. A Chinese study done in 2016 in a similar setting showed a prevalence of 15-40%.[10] Not many studies have calculated the overall prevalence of gonadal tumors among the Y chromosome containing DSD subjects. The prevalence of gonadal tumor in subjects with gonadal dysgenesis and androgen insensitivity syndrome has been delineated by Cools *et al*.[9] On analyzing the subset group, we could find that the estimated prevalence of GCT's among MGD was 20%; which is comparable to previous studies (15 to 18%).[11-16] The prevalence of GCT's in CGD in our study was 20% which was similar to the reports from previous studies, i.e., 15-30%.[17] But the prevalence of GCT's among CAIS and PAIS was 25% and 19.2% in our series compared to the earlier studies on AIS which was 0.8% and 15% respectively.[9] This higher prevalence may be attributed to the higher proportion of post-pubertal patients among the AIS group in our study. Previous studies have shown that the risk of gonadal tumors rose after puberty among the AIS subjects (as much as 3.6% and 33% at 25 and 50 years respectively).

The prevalence of malignant gonadal GCT in our study ranged from 7.7 to 21.1%, the highest occurred among PAIS subjects followed by MGD and CGD. CAIS had the least risk of gonadal malignancy ($P < 0.01$). We learned that this is the first study to do a head to head comparison of malignant gonadal tumor among MGD, CGD, CAIS, and PAIS. The higher preservation of germ cell mass at puberty in PAIS (up to two-third of germ cell mass is preserved) may be one possible explanation for the higher malignancy rate.[18,19] Germ cells are essential for the clonal expansion and later tumorogenesis.[20] Androgens promote germ cell proliferation, mediated via mature Sertoli cells which express androgen receptors only around and after puberty.[21] Though AIS is characterized by deficient androgen signaling due to resistance at the receptor level, germ cell proliferation under the effects of androgen occurs in varying degrees in PAIS after puberty. Moreover in PAIS, the defects underlying this syndrome may develop from a wide range of genetic mutations that are not completely understood, suggesting the possibility of developing malignancy in this particular DSD may be higher than previously known. It is very important to be aware of the findings associated with AIS, which may be undiagnosed and drastically change the fate of patients. In gonadal dysgenesis, subjects show testicular tissue with variable degrees of dysgenesis, containing germ cells that abundantly express testis-specific protein on the Y chromosome (TSPY), survives and proliferates in an unfavorable environment, and later form GCT's.[22,23] In CAIS subjects there is a rapid and complete loss of germ cells, starting as early as one year of age and hence possibly at lower risk to develop malignant GCT's compared to PAIS and gonadal dysgenesis. Premalignant lesions (CIS and gonadoblastoma) were found in a prevalence of 5.0 to 18.75%. The most common gonadal tumors found, were dysgerminoma (29.4%) followed by seminomas and mixed GCT (each 17.6%), gonadoblastoma (11.8%), and yolk sac tumor (5.8%) being the rarest. Among the gonadal dysgenesis (MGD and CGD)

Table 2: The overall prevalence of gonadal GCT among the study subjects [Gonadal samples (S) = 82]

	MGD (S=20)	CGD (S=20)	CAIS (S=16)	PAIS (S=26)	Total (S=82)
No Risk	16	16	12	21	65
Gondal GCT	4	4	4	5	17
Overall risk$	4/20 (20%)	4/20 (20%)	4/16 (25%)	5/26 (19.2%)	17/82 (20.7%)

*Overall tumor risk between the groups was calculated and found to be significantly different (P<0.01)

Table 3: Characteristics of germ cell tumors among the DSD subjects with the Y chromosome

Types of DSD	Total subjects *n*=54	Number of gonadectomy samples (S=82)	Age of tumor diagnosis years (median)	Number of GCT	Gonadoblastoma (2)	CIS (3)	Dysgerminoma (5)	Seminoma (3)	Yolk sac tumor (1)	Mixed GCT (3)
MGD	10	20	17	4	1	0	2	0	0	1
CGD	10	20	16.5	4	1	0	2	0	0	1
CAIS	8	16	24	4	0	3	1	0	0	0
PAIS	26	26	18	5	0	0	0	3	1	1

The distribution of malignant gonadal tumors in the descending order of occurrence in the subjects were dysgerminoma (*n*=5; 29.4%), seminoma (*n*=3, 17.6%), mixed GCT (*n*=3, 17.6%) and yolk sac tumor (*n*=1, 5.8%)

Table 4: Multivariance logistic analysis showing the odds of occurrence of gonadal GCT among DSD subjects with Y chromosome based on risk markers (age at or above 18 years, presence of congenital anomalies, and EMS score 7 or above)

Logistic analysis	Variables	Odds ratio	95% CI	*P*
	Age >18 years	7.34	3.42-15.76	<0.01
	Presence of congenital anomaly	3.09	1.69-5.65	<0.01
	EMS	1.18	0.32-4.31	0.89

The multivariance logistic regression analysis showed that age at or above 18 years [OR 4.23 (CI 0.84-12.26) *P*<0.01] and presence of congenital anomalies like renal agenesis, congenital diaphragmatic hernia, and retroperitoneal vascular defects [OR -4.63 (CI 1.27-16.75), *P*<0.01] are independently associated with the occurrence of gonadal GCT's among the study subjects

dysgerminoma was the most common type of GCT. Among the PAIS patients, the most common GCT was seminoma. The most infrequent GCT was yolk sac tumors. CAIS patients had the highest number of CIS. Only one case of CAIS was detected to have dysgerminoma at the age of 34. Malignant GCT is extremely rare in CAIS as explained above though the incidence of malignant GCT increases as the age progresses.[9,10] Some authors have questioned the need for gonadectomy among the CAIS considering the low or absent risk of developing malignant GCT.[24] Though rare, we would like to emphasize that the chance of developing a malignant GCT is not an absolute zero in CAIS.[10] The role of genotypes may also influence tumorigenesis. The time interval between the progressions from CIS to invasive GCT has not been studied yet. In this context, it would be justified to undergo gonadectomy after completing puberty among CAIS. The role of genotypes may also influence tumorigenesis.

We also embarked to find the risk markers for predicting the occurrence of gonadal tumor. We divided our subjects into the pediatric group (age less than 18 years) and adults (age equal and more than 18 years). We found that the tumor risk among the study subjects above 18 years (28.6%) was significantly higher compared to the study subjects below 18 years (12.5%) ($P < 0.01$). The risk was twice the number in the adult subjects. The same trend could be seen in each (MGD, CGD, and PAIS) group ($P < 0.01$). The possible explanation is as the age advances the neoplastic initiation may be induced by multiple mutations of related genes (Knudson hypothesis).[25] A risk of 0.8–16% has been mentioned by a few authors.

Apart from age, other risk markers studied were EMS and associated congenital anomaly. It is already known that an abdominal or inguinal position of the gonad represents an additional independent risk factor for malignant change and the same was evident here.[24,26] We divided EMS into two categories based on the score. EM score 7 – 12 was considered mildly virilized and less than 7 under virilized. Though chances of gonadal tumors among the mildly virilized DSD subjects were higher compared to under virilized subjects ($P < 0.01$), multivariance analysis didn't show so. We also found that patients with DSD with Y chromosomes had a congenital diaphragmatic hernia, unilateral renal agenesis, and abnormal vasculature (left-sided IVC). The association of these congenital anomalies and 46 XY DSD has been reported. The known associations are Denys-Drash syndrome with congenital diaphragmatic and renal abnormalities with mixed gonadal dysgenesis as a part of Turner's syndrome.[27,28] Abnormal vascular developments have been described in Turner syndrome. In our study, left-sided inferior vena cava was seen only in the case of CGD and PAIS. Such association is first to be described in 46 XY DSD. Peter *et al.* have described the association between retroperitoneal vascular abnormalities and renal agenesis with testicular germ cell tumors in men.[29] The link between maldescent testis and testicular tumors has been voiced. It seems that an insult occurring during fetal testicular descent also includes an effect on the embryogenesis of other organs; particularly the vascular and renal system could contribute to the spectrum of retroperitoneal anomalies. Cryptorchidism (nine times) were previously reported

to be a risk factor for testicular carcinoma.[29-31] Connecting all the above information it can be inferred that there is an inter-relationship between undescended testis, retroperitoneal anomalies, and testicular germ cell tumors. The presence of congenital anomalies was significantly higher among gonadal tumors in the study ($P < 0.01$). Multivariance logistic analysis showed the presence of congenital anomalies and age at or more than 18 years to be considered as an independent risk marker for the occurrence of gonadal tumors in DSD patients with Y chromosomes [Table 4]. The limitation of our study was the small sample size. Secondly, subjects were not uniformly screened for gonadal malignancy in each group. Gonadectomy and detection of malignancy were done for different clinical situations and considering the nature of the disease and its medical aspects we accept this as a major limitation. Also, none of the scrotal testis among the PAIS had a biopsy was done which could have helped to compare the cancer risk of scrotal vs non-scrotal testis which is still unknown.

Conclusion

The prevalence of gonadal GCT's among DSD with Y chromosomes is nearly 25%. Dysgerminoma is the most common malignant gonadal tumors seen. PAIS had the maximum risk of developing malignant gonadal GCT's. Age above 18 years and the presence of congenital anomalies like renal agenesis, retroperitoneal vascular defects, and congenital diaphragmatic hernia are independent risk markers for the development of gonadal GCT's.

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Conflicts of interest

There are no conflicts of interest.

References

1. Oosterhuis JW, Looijenga LH. Testicular germ-cell tumors in a broader perspective. Nat Rev Cancer 2005;5:210-22.
2. Oosterhuis JW, Stoop H, Dohle G, Boellaard W, Van Casteren N, Wolffenbuttel K, *et al*. A pathologist's view on the testis biopsy. Int J Androl 2011;34:e14-9.
3. Piazza MJ, Urbanetz AA. Germ cell tumors in dysgenetic gonads. Clinics (Sao Paulo) 2019;74:e408.
4. Lee PA, Nordenstrom A, Houk CP, Ahmed SF, Auchus R, Baratz A, *et al*. Global disorders of sex development update since 2006: Perceptions, approach, and care. Horm Res Paediatr 2016;85:158–80.
5. Goyal A, Kubihal S, Gupta Y. Dynamic testing for evaluation of adrenal and gonadal function in pediatric and adult endocrinology: An overview. Indian J Endocr Metab 2019;23:593-601.
6. Ahmed SF, Khwaja O, Hughes IA. The role of a clinical score in the assessment of ambiguous genitalia. BJU Int 2000;85:120–4.
7. McCann-Crosby B, Gunn S, Smith EO, Karaviti L, Hicks MJ. Association of immunohistochemical markers with premalignancy in Gonadal Dysgenesis. Int J Pediatr Endocrinol 2015;2015:14.
8. Elbe JN, Sauter G, Epstein JI, Sesterhenn IA. Pathology and Genetics of Tumors of the Urinary System and Male Genital Organs. Lyon, France: IARC Press; 2004.
9. Cools M, Drop SL, Wolffenbuttel KP, Oosterhuis JW, Looijenga LH. Germ cell tumors in the intersex gonad: Old paths, new directions, moving frontiers. Endocr Rev 2006;27:468-84.
10. Huang H, Wang C, Tian Q. Gonadal tumour risk in 292 phenotypic female patients with disorders of sex development containing Y chromosome or Y-derived sequence. Clin Endocrinol (Oxf) 2017;86:621-7.
11. Manuel M, Katayama PK, Jones HW Jr. The age of occurrence of gonadal tumors in intersex patients with a Y chromosome. Am J Obstet Gynecol 1976;124:293–300.
12. Robboy SJ, Miller T, Donahoe PK, McCullough LB, Sutton VR, Austin EG, *et al*. Dysgenesis of testicular and streak gonads in the syndrome of mixed gonadal dysgenesis: Perspective derived from a clinicopathologic analysis of twenty-one cases. Hum Pathol 1982;13:700–16.
13. Gourlay WA, Johnson HW, Pantzar JT, Pantzar JP, Barbara M, Richard C. Gonadal tumors in disorders of sexual differentiation. Urology 1994;43:537–40.
14. Ramani P, Yeung CK, Habeebu SS. Testicular intratubular germ cell neoplasia in children and adolescents with intersex. Am J Surg Pathol 1993;17:1124–33.
15. Saikia UK, Sarma D, Das DV, Goswami JK, Kaushik, Saikia C, *et al*. A case of mixed gonadal dysgensis: A diagnostic challenge. J Hum Reprod Sci 2019;12:169-72.
16. Cools M, Pleskacova J, Stoop H, Hoebeke P, Van Laecke E, Drop SL, *et al*. Gonadal pathology and tumor risk in relation to clinical characteristics in patients with 45, X/46, XY mosaicism. J Clin Endocrinol Metab 2011;96:E1171–80.
17. Michala L, Goswami D, Creighton SM, Conwaya GS. Swyer syndrome: Presentation and outcomes. BJOG 2008;115:737–41.
18. Cools M, van Aerde K, Kersemaekers AMF, Boter M, Drop SL, Wolffenbuttel KP, *et al*. Morphological and immunohistochemical differences between gonadal maturation delay and early germ cell neoplasia in patients with undervirilization syndromes. J Clin Endocrinol Metab 2005;90:5295–303.
19. Cools M, Wolffenbuttel KP, Drop SL, Oosterhuis JW, Looijenga LH. Gonadal development and tumor formation at the crossroads of male and female sex determination. Sex Dev 2011;5:167–80.
20. Rey RA. Mini-puberty and true puberty: Differences in testicular function. Ann Endocrinol (Paris) 2014;75:58–63.
21. Hannema SE, Scott IS, Hodapp J, Martin H, Coleman N, Schwabe JW, *et al*. Residual activity of mutant androgen receptors explains wolffian duct development in the complete androgen insensitivity syndrome. J Clin Endocrinol Metab 2004;89:5815–22.
22. Schellhas HF. Malignant potential of the dysgenetic gonad. Part 1. Obstet Gynecol 1974;44:298–309.
23. Uehara S, Hashiyada M, Sato K, Nata M, Funato T, Okamura K. Complete XY gonadal dysgenesis and aspects of the SRY genotype and gonadal tumor formation. J Hum Genet 2002;47:279–84.
24. Cools M, Wolffenbuttel KP, Hersmus R, Mendonca BB, Kaprová J, Drop SLS, *et al*. Malignant testicular germ cell tumors in postpubertal individuals with androgen insensitivity: Prevalence, pathology, and relevance of single nucleotide polymorphism-based susceptibility profiling. Hum Reprod 2017;32:2561–73.
25. Cools M, Stoop H, Kersemaekers AM, Drop SL, Wolffenbuttel KP, Bourguignon JP, *et al*. Gonadoblastoma arising in undifferentiated gonadal tissue within dysgenetic gonads. J Clin Endocrinol Metab 2006;91:2404–13.
26. Cortes D, Thorup J, M, Visfeldt J. Cryptorchidism: Aspects of fertility and neoplasms. Horm Res 2001;55:21-7.
27. Esplin ED, Chaib H, Haney M, Martin B, Homeyer M, Urban AE, *et al*. 46, XY disorders of sex development and congenital diaphragmatic hernia: A case with dysmorphic facies, truncus arteriosus, bifid thymus, gut malrotation, rhizomelia, and adactyly. Am J Med Genet A 2015;167:1360–4.
28. Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME,

Klein KO, *et al*. Clinical practice guidelines for the care of girls and women with Turner syndrome: Proceedings from the 2016 Cincinnati international Turner syndrome meeting. Eur J Endocrinol 2017;177:G170.
29. Holt PJ, Adshead JM, Filiadis I, Christmas TJ. Retroperitoneal anomalies in men with testicular germ cell tumors. BJU Int 2007;99:344–6.
30. Das DV, Jabbar PK, Gomez R, Seena TP. Hemoptysis: A rare presentation of mixed gonadal dysgenesis. J Hum Reprod Sci 2020;13:242-4.
31. Das S, Saikia UK, Saikia KK, Sarma D, Choudhury BK, Bhuyan AK, *et al*. Spectrum of 46 XY disorders of sex development: A hospitalbased crosssectional study. Indian J Endocr Metab 2020;24:360-5.