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A 10-YEAR STUDY OF CHILDREN WITH GONADAL TUMORS AND DISORDERS OF SEX DIFFERENTIATION, IN ROMANIA

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Abstract

Context. Children having gonadal tumors and disorder of sex differentiation (DSD) are rare.

Objective. To investigate the presentation of DSD children with malignant gonadal tumors.

Methods. A retrospective study from 2010-2020, that evaluated 17 children with DSD, including 13 females, eight months to 16 years, with congenital adrenal hyperplasia, 5-alpha reductase deficiency, androgen insensitivity syndrome, Turner, Sywer, and Klinefelter syndromes.

Results. Ten children had malignant gonadal tumor; nine had germ cell tumors and one person granulosa cell tumors, while seven children with non-malignant tumor had gonadoblastoma, cystadenoma (five children), and cysts. Systemic malformations, obesity, elevated tumor markers, and psychosocial issues were observed in 90%, 90%, 70%, and 50% of children with malignancy unlike 28.6%, 42.9%, 14.35%, and 57.1% children without malignancy respectively. Most (9/10) children >12 years, had psychosocial issues, unlike 0/7 children ≤12 years. From 8/17 children presenting with symptoms suggestive of tumor, 75% had malignancy, while from 9/17 children with DSD presentation, 44% had malignant tumors. Malignancy was observed in 3/10 children between eight months to age six, while 7/10 children had stage 1-2 tumors. We reported a child, identified as female, aged 13 years, with partial androgen insensivity syndrome (PAIS) 46,XY, and testicular papillary serous cystadenoma with genomic variant AR NM_000044.4:c.2750del. p.(F917Sfs*27) chromosome Xq12, never published in people with PAIS nor population databases (GnomAD).

Conclusion. DSD diagnosis raises numerous challenges. People with DSD have increased risk of malignancy, especially when obesity and, systemic malformations are present; also, psychosocial issues in these children are associated with postpubertal age.

Keywords: Disorders of sexual differentiation, testicular tumors, ovarian tumor, children, partial androgen insensitivity syndrome.

INTRODUCTION

Gonadal tumors are complex neoplasms arising from the testis or ovary, rarely found in children, have incidence rates of 2.6:100000 and 1-12:100000 respectively, with a higher chance of cure, when discovered early (1, 2). Disorders of sex development (DSD) are also rare, complicated, and delicate conditions where different phenotypic sex anatomical structures, gonadal, or genetic chromosomal are atypically found in the same individual, having an incidence rate of 1:5,000 live births (3). Studies revealed an increased risk of gonadal malignancy in children is associated with congenital malformation (non-chromosomal or chromosomal related, including genital organ abnormality related to DSD), and the risk increases with the number of malformations, more in children than adults (4, 5). Also, a multidisciplinary team is needed to accurately diagnose and manage children with gonadal tumors and DSD (6).

There are risks of malignancy in people with DSD if, during embryogenesis immature germ cells persist, also in the presence of histological dysgenetic gonad development, gonadoblastoma (+Y), intrabdominal location of the gonads, OCT3/4 (+) marker, nonscrotal partial androgen insensivity syndrome (PAIS), Frasier, Denys-Drash (+Y), Intermediate Turner (+Y), 12[th] and 9[th] chromosome gain, aneuploidy, loss of 6q and elevated tumor markers (7, 8).

The 2006 consensus shows the diverse

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classification of DSD (8) caused by chromosomal disorders, reduced testosterone secretion, impaired testosterone action, incomplete virilization, or high androgen level exposure at the prenatal period (9). Prophylactic gonadectomy is one of the treatment management for children with DSD, with a high risk of developing malignant gonadal tumors (7). Partial Androgen Insensitivity Syndrome (PAIS), with an incidence of 5–7:1,000,000 in genetic males, is an X- linked recessive inherited disease that occurs due to partially unresponsive cells to androgen caused by Androgen Receptor (AR) gene dysfunction, causing male, ambiguous, or female genitalia (10, 11). Additionally, testicular epithelial tumor in children are rarely reported, as 95% of testicular tumors are germ-cell tumors (12). We report the study of children with DSD and gonadal tumors, with details on a rare case of a girl with PAIS and testicular papillary serous cystadenoma.

Aim

To investigate the presentation of DSD children with malignant gonadal tumors.

MATERIALS AND METHODS

Participants

We carried out a 10-year retrospective study (2010-2020), in 3 hospitals: the Emergency children's hospital in Iasi, Bacau, and Timisoara, Romania. Using the inclusion criteria of - DSD children till age 17 having gonadal tumors, from 210 children with gonadal tumors we identified 17 children with DSD, from eight months to 16 years, with malignant tumors: seminoma, yolk sac tumor, dysgerminoma, immature teratoma, Granulosa cell tumors, mixed Germ cell tumors (GCTs); and benign tumors: gonadoblastoma, cysts, serous, mucinous and borderline cystadenoma. The collected data from the children's medical files included: diagnoses, age, sex, BMI, laboratory results of estradiol, total testosterone, luteinizing hormone, antimullerian hormone, follicle-stimulating hormone, and tumor markers, Lactate Dehydrogenase (LDH 0-300u/L), Beta-Human Chorionic Gonadotropin (β-hCG-5.3IU/L), cancer antigen 125 (Ca125 -0-35u/L) and Alpha-Fetoprotein (AFP- 9.92IU/mL), treatment, and malformations. Puberty was assessed according to the Tanner staging system. Psychosocial issues in this study refers to difficult happening in the life of an individual, like loss, abuse, financial distress, linked to occurrence of anxiety, depression, or an affected mental health in general (13). For the cytogenetic study, standard Giemsa-banding culture was used, and fluorescence *in-situ* hybridization test for sex-determining region of the Y (SRY) on Yp11.3 region, using the locus specific-SRY probe. Next-generation sequencing TruSight One Illumina Gene Panel Sequencing was used for further molecular genetic analysis (14, 15). The histopathology study was performed by expert pathologists who examined the gonadal specimen to identify the histological component. Diagnoses were determined based on multiple representative slides (six and above), using hematoxylin and eosin stain on a Leica DM 750 Optical microscopes (Leica Biosystem Newcastle Ltd, New Castle Upon Tyne NE 12 EW, United Kingdom), Leica AirLab Microsystems GmbH and immunohistochemical analysis were performed to confirm malignancy, including OCT3/4 marker. For the statistical study we analyzed our data using Stata, version 17 (2021) Stata Corp, TX, USA; IBM SPSS version 16.0 (Windows), USA for logistic regression for relative risk ratio (RR), 95% Confidence interval (CI), Chi-square test, sensitivity, specificity and, statistically significant results had P values <0.05.

Ethical Statement

This study's ethical approval was obtained from the ethics committee of the Victor Babes University of Medicine and Pharmacy, Timisoara, and the ethics committee of the Emergency Hospital for Children Iasi, Bacau, and Timisoara. Nr. 59/12.12.2018, 123/2020 and 11.12.2020 respectively. This study has complied with relevant national regulations, institutional policies and in accordance with the tenets of the Helsinki Declaration. Written and informed consents were obtained from parents of each child who participated in the study.

RESULTS

In this study, 210 children had gonadal tumors, from which 17 (8.1%) children were identified with DSD. These children's demographic characteristics, DSD genetic diagnoses, presentations, malformations, treatment, presence of psychosocial issues, tumor type and stage, tumor marker, hormonal levels, and gonadal tissue observed are presented in Table 1. Ten children had malignant tumors with dysgerminoma (40%) mainly observed, while amongst the seven children with non-malignant tumors, cystadenoma (71.4%) was majorly noted. One non-malignant tumor case was already discussed (16). Most children with DSD, in this cohort,

were assigned as females at birth (76.5%). 8/17 children had gonadal tumors presentations, and on examination, DSD was identified, from which 75% were children with malignant tumors. Conversely, nine children that presented with DSD complaints, were later diagnosed with gonadal tumors, this included five children with non-malignant tumors. 82% of our participants were diagnosed at puberty, 30% of participants who had malignant tumors were at prepuberty age, while 70% of participants with malignancy at puberty. We observed in the children with malignancy, 7/10 had tumor stages 1 and 2. A 14-year-old girl with PAIS, and dysgerminoma, stage 4 died after three years from diagnosis.

Obesity was observed in 3/7 children (42.9%) with non-malignancy, unlike 9/10 children (90%) with malignancy p-value 0.036. Tumor markers AFP (Immature teratoma, yolk sac tumor, mixed GCT), B-HCG (seminoma, mixed GCT), LDH (seminoma, mixed GCT, two immature teratomas, yolk sac tumor, two dysgerminomas), and Ca-125 (Dysgerminoma, immature teratoma) were elevated in 70% of children (7/10) with malignancy and 14.3% of children (1/7) without malignant tumors (borderline cystadenoma-LDH, Ca-125) P-value 0.023. We observed OCT ¾ markers was used in few children (five cases- 29%), as immunohistochemistry was performed to confirm malignancy. Three children with seminoma and two dysgerminoma had positive findings, while immunohistochemistry was negative in two children with yolk sac tumors and mixed GCT. Malformations in other systems were observed in 9/10 children (90%) with malignancy in contrast to 2/7 children (28.6%) without malignancy p-value 0.001. Psychosocial issues were observed in four children (57%) and absent in three children (43%) with non-malignant tumors ages ≤12 years, and absent in five children having malignancy, with 4/5 (80%) of ages ≤10 years. In total, psychosocial issues were observed in 9/10 children (90%) >12 years, compared to 0/7 children (0%) ≤12 years, P-value 0.001. Gonadectomy in agreement with the choice of the children's family, was performed in two non-malignant tumor children (22%), compared to all children (100%) with malignancy P-value 0.001. From the 17 children with DSD, the sensitivity in identifying testicular tissues for total testosterone, and AMH was 72.7%, and 63.3%, while the specificity was 83.3% and 80% respectively, using IBM SPSS version 16.0. Also using the normal ranges of total testosterone, LH, FSH, estradiol, and AMH, an alteration in these ranges to hint DSD diagnoses and their sensitivity was observed in 13 children 76.5%, 14 children 82.4%, eight children 47.1%, five children 29.4% and 14 children, 87.5% respectively. Although the sensitivity of testosterone, estradiol, and AMH, will be increased to 90%, 64.7%, and 93.8%, if we consider children having a very low normal range, in order to further examine them and rule out DSD, also recognizing that hormonal results overlap is possible. Amongst the children with benign tumors, 6/7 (85.7%) has testicular tissue, while amongst the children with malignant tumors, 7/10 (70%) had testicular tissues and streak gonads. The median and range values for testosterone, LH, FSH, estradiol, and AMH for benign tumors were 1(0.08-13) ng/ml, 24 (12-48) UL/L, 6 (2-68.2) UL/L, 9.7(6-40.5) pg/ml, 225.5(0.36-427), while for malignant tumor it was 0.51(0.026-11) ng/ml, 14.3 (1-37)UL/L, 5.25 (3-55) UL/L, 12(1-30) pg/ml, 2(0-300) ng/ml. P- values 0.001. Also, we noted that amongst the two children with 5-alpha reductase deficiency, AMH was elevated in the child with granulosa cell tumor (13ng/ml), unlike the other child with immature teratoma (3.2ng/ml).

From the 210 children with gonadal tumors, the univariate relative risks of malignancy in children with DSD (17 cases), p-value, and 95% confidence interval in comparison to the multivariate relative risk , analyzing also other risk factor including; obesity, maternal age, breastfeeding ≤5 months, stress, hormone, smoking, positive heredo-genetic history, abnormal birth weight, residence area, pollution, rhesus positive, menstruation disorders, malformations, urinary tract infection, no postnatal vitamin D and vaccine, is presented in Table 2. A particular case of a girl aged 13, presented with the complaint of primary amenorrhea. Her maternal aunt had the same history, being a carrier of Androgen insensitivity syndrome (AIS), and died of an unknown cause. On physical examination: she has a broad chest, 158cm tall, Tanner index: pubic hair stage 2, breast stage 3, flat nipples, sparse axillary, and mild clitoromegaly, blind vagina: 2,5cm length near the urethral opening and minimal labia adhesion posteriorly; Prader stage 2. Hormonal analysis revealed: ↑Testosterone: 4.4ng/ml, Dihydrotestosterone: 221pg/ml, FSH: 2.73mUL/ml, ↑LH:30.3mUl/ml, ↑AMH: 427.260ng/ml, ↓Estradiol: 6pg/ml, Progesterone: 0.33ng/ml, 5α-reductase and 17β-hydroxysteroid dehydrogenase deficiencies were excluded. LDH, Ca125, B-HCG, and AFP were normal. Her chronological age correlated to her bone age, and a female identity was attested, with an intelligence quotient score of 74- borderline. Cytogenetic analysis, using standard culture Giemsa-banding, showed 46,XY

Table 1. The clinical data of the children with disorder of sex differentiation and gonadal tumors in the study (* Low but in normal limit, ** Laboratory result done at 11 years and 6 months)

S/N	DSD Diagnoses	Sex	Age	Benign Tumor	Tumor Stage	Elevated tumor marker	Malformation	Obesity
1	Turners Syndrome (45X/46XY Mixed gonadal dysgenesis)	F	13	Gonadoblastoma	No	No	Short, short neck and delay hands bone age	Yes
2	Klinefelter (XXY)	M	15	Seros cystadenoma	No	No	No	No
3	PAIS (46XY)	F	10	Mucinos cytsadenoma	No	No	No	yes
4	PAIS (46XY)	F	13	Papillary Seros cystadenoma	No	No	Nasal septal deviation	No
5	Klinefelter (XXY)	M	12	cyst	No	No	No	yes
6	CAIS (46XY)	F	11	Mucinos Borderline cystadenoma	No	Yes	No	No
7	PAIS (46XY)	F	14	Mucinos cystadenoma	No	No	No	No
S/N	DSD Diagnoses	Sex	Age	Malignant Tumor	Tumor Stage	Elevated tumor marker	Malformation	obesity
1	Turners Syndrome (45X)	F	10	Dysgerminoma	1	Yes	Short, short neck, hydronephrosis	yes
2	Sywer Syndrome (46XY)	F	1	Yolk	1	Yes	Strabismus, congenital inguinal hernia	No
3	CAIS (46XY)	F	<1	Dysgerminoma	1	No	Septal nasal defect	Yes
4	Turners Syndrome (45X)	F	13	Dysgerminoma	3	Yes	Short, short neck, Scoliosis	Yes
5	Klinefelter (XXY)	M	16	Seminoma	1	Yes	Choledochal cyst	yes
6	Sywer Syndrome (46XY)	F	6	Mixed GCT	2	Yes	Short, strabismus	Yes
7	5-alpha reducta se deficiency (46XY)	F	14	Granulosa cell tumors	2	No	Thyroid dysgenesis	Yes
8	PAIS (46XY)	F	14	Dysgerminoma	4	No	Hydronephrosis, astimatization (myopic)	Yes
9	5-alpha reductase deficiency (46XY)	F	15	Immature Teratoma	2	Yes	short neck, cleft palate, retrognathia, short, down syndrome, tracheomalacia, atrial septal defect, strabismus, Clubfoot	Yes
10	congenital adrenal 21 hydroxlase (46,XX)	M	13	Immature Teratoma	3	Yes	No	yes

karyotypes (Fig. 1a). Fluorescence *in-situ* hybridization test revealed that the SRY gene is present in the Yp11.3 region, using the locus-specific-SRY probe. The sequencing and karyotyping classic assessments method were performed in Romanian Regional Centers of Medical Genetics Timis, part of the European Reference Network ITHACA. Next-generation sequencing utilizing MiSeq machine (Illumina, San Diego, USA) and TruSightOne gene panel (4813 genes, including AR gene) was performed. Bioinformatics was explained previously (23-24). The result showed a likely pathogenic, frameshift (null variant), gene- AR OMIM 313700. PAIS (OMIM 300068), transmission X-linked. NM_000044.4:c.2750del, p.(F917Sfs*27),

Gonadectomy	Psychosocial issues	Presentation	Gonadal tissue	Total Testosterone(ng/ml)	LH (UL/L)	FSH (UL/L)	Estradiol (pg/ml)	AMH (ng/ml)
No	Yes	Short	Ovary	**0.33 (0.26-1.17)	**12.6 (<0.09-14.36)	**68.2 (0.05-7.92)	**<10 (10-117.44)	n.a
yes	yes	Micro-penis, cryptorchidism, Gynecomastia	Testis	0.9 (1-12)	24 (0.8-8.7)	30 (0.6-6.9)	40.5 (≤38)	0.36 (<13)
No	No	Ambiguous genitalia	Testis	1(<0.07-0.44)	15 (<0.02-4.8)	2 (0.5-6.0	*7.2 (≤24)	350 (0.36-5.9)
No	yes	Amenorrhea	Testis	4.4(<0.07-0.75)	25.5 (<0.02-11.7)	2.26 (0.9-8.9)	6 (15-85)	427 (0.49-6.9)
No	No	Micropenis, cryptorchidism	Testis	0.08(<0.07-8)	12 (0.1-5.7)	15 (0.6-6.9)	19 (≤16)	1.1 (<13)
yes	No	Abdominal pain	Testes	2(<0.07-0.44)	48 (<0.02-11.7)	6 (0.9-8.9)	*9.7 (≤60)	201 (0.36-5.9)
No	Yes	Abdominal mass	Testis	13(<0.07-0.75)	30 (<0.02-16.7)	2.7 (0.9-8.9)	16.4 (15-350)	250 (0.49-6.9)
Gonadectomy	Psychosocial issues	Presentation	Gonadal tissue	Total Testosterone(ng/ml)	LH (UI/L)	FSH (UI/L)	Estradiol (pg/ml)	AMH (ng/ml)
yes	No	Short,	Ovary	*0.07(<0.07-0.44)	21 (<0.02-4.8)	43 (0.5-6.0)	*8 (≤24)	1 (0.36-5.9)
Yes	No	Abdominal pain, mass	Streak gonads	0.04(<0.07-0.20)	1 (<0.02-0.3)	6.4 (0.5-6.0)	*5.2 (≤20)	Undetected (0.11-4.2)
Yes	No	Abdominal pain	Testis	0.3(<0.07-0.20)	20 (<0.02-18.3)	3 (1.2-12.5)	*6.5 (≤20)	278 (0.11-4.2)
Yes	yes	Abdominal pain	Ovary	0.19 (<0.07-0.75)	26 (<0.02-11.7)	55 (0.9-8.9)	10 (15-85)	0.4 (0.49-6.9)
yes	yes	Micropenis, Gynecomastia	Testis	0.72 (1-12)	30 (0.8-8.7)	41 (0.7-9.6)	30 (≤38)	*0.6 (<13)
yes	No	Abdominal mass	Streak gonads	0.03(<0.07-0.20)	1.1 (<0.02-0.3)	20 (0.5-6.0)	*5.9 (≤20)	Undetected (0.21-4.9)
Yes	yes	Amenorrhea. hypothyroidism	Testis	4.4(<0.07-0.75)	7 (<0.02-16.7)	3.2 (0.9-8.9	20 (15-350)	13 (0.49-6.9)
Yes	No	Abdominal mass	Testis	11 (<0.07-0.75)	37 (<0.02-16.7)	4 (0.9-8.9)	15 (15-350)	300 (0.49-6.9)
Yes	yes	Amenorrhea, micropenis	Testis	5.8(<0.07-0.75)	8.6 (<0.02-16.7)	4.5 (0.9-8.9	16.2 (15-350)	3.2 (0.62-7.8)
yes	yes	Abdominal pain,	Ovary	10 (<0.07-8)	2.5 (0.1-5.7)	3.9 (0.6-6.9)	14 (≤26)	*3 (<13)

Hemizygous. Where loss-of-function is a recognized disease's mechanism, associated with partial androgen insensitivity. To the best of our knowledge the variant was not published in literature in persons with AIS nor present in population databases (GnomAD). The variant was classified with likely pathogenic significance according to the American College of Medical Genetics and Genomics guidelines (17). Ultrasound and abdominal-pelvic magnetic resonance imaging revealed intra- abdominal testis 18/12mm left and 24/40mm right (Figs 1b,1c), the absence of mullerian structures, and a vagina was not observed. Based on the parents' wish, only exploratory laparoscopy, cystoscopy, vaginoscopy, and biopsy were performed,

which confirmed the presence of testes, a blind end vagina, and absence of the uterus, ovaries, and fallopian tubes (Fig. 2a). The pathology result revealed testicular papillary serous cystadenoma (Figs 2b,2c,2d).

We observed that the child and parents accepted the sex assigned from birth, and after discussion with the family, the recommended treatment included estradiol, 40µg/24h, I/2 patch/week, transdermal, bi-annual endocrinological re-evaluation, and regular psychological counseling. After twelve months of estradiol treatment, the child looked more feminine, with Tanner index, breast stage 4, pubic hair stage 3. The organization and communication between the multidisciplinary team from different departments were challenging. The child attempted suicide four months after the initial presentation, ingesting metoclopramide in overdose. The parents and child had anxiety in regards to the tumor progression, to being rejected in a relationship, peer pressure, and especially when occurring at puberty, a stage of increased consciousness

Table 2. Relative risk of malignancy in children with disorder of sex differentiation

Number of children with DSD having gonadal tumor	Univariate relative risk	p-value	95% confidence interval	Multivariate relative risk	p-value	95% confidence interval
17/210	1.42	0.104	0.93-2.16	0.79	0.179	-0.356 -1.908

Table 3. Published studies of children with disorder of sex differentiation and gonadal tumor

S/N	Diagnoses	Total number of cases	Age (years)	Sex	Malformation	Tumor	Gonadectomy	Supports prophylactic gonadectomy
Niu HL *et al.* 2021 (25)	46XY, 45X, DSD	6	≤13	4F 2M	No	Gonadoblastoma, GCNIS, Mixed GCT, Seminoma, Dysgerminoma	N/A	N/A
Vogt PH *et al.* 2019 (26)	46XY	2	6,16	F	Denys Drash	Gonadoblastoma, Dysgerminoma	No	N/A
Faure-Conter C. 2020 (27)	46XX/45XO, 46XY/45XO, Klinefelter 46XY	16	M (14 median) F (15 median)	5M, 11F	Poly-malformative syndrome	GCT, Gonadoblastoma	N/A	N/A
Racoma MJC *et al.* 2022 (28)	45X/46XY	1	13	F	Short	Dysgeminoma	Yes	Yes
Steinmacher S. *et al.* 2021 (29)	CAIS, PAIS, 17-β-hydroxy steroid dehydrogenase deficiency, homozygous LH-receptor deficiency XY-DSD	17	≤17	F	No	Benign	Yes	Only at child's wish
Fei YF. *et al.* 2023 (30)	46 XY, 45X/46XY, Partial gonadal dysgenesis, ovotesticular, CAIS	70	≤17	N/A	No	Gonadoblastoma, Dysgerminoma	Yes	Yes
Matsumoto F *et al.* 2020 (31)	45X/46XY	7	Children	1M, 6F	No	Gonadoblastoma, Seminoma, Dysgerminoma	Yes	Yes
Bailez MM. *et al.* 2019 (32)	46XY, 46XX	15	Neonate	N/A	No	GCT, Gonadoblastoma	Yes in some children	Only in streak gonads
Norton JC. *et al.* 2021 (33)	46XY, CAIS	1	15	F	Congenital abnormalities	GCNIS, Leiomyoma	Yes	Yes
Das D. 2021 (34)	45X/46XY	1	6	F	Short, skeletal	Dysgerminoma	Yes	Yes

of body development in children, all these influenced the management process.

DISCUSSION

Gonadectomy is one of the treatment options for DSD because of the possibility of the occurrence of gonadal tumors due to their similar etiopathogenesis: abnormal differentiation of totipotential cells, metaplasia of the tunica vaginalis' mesothelial, or incomplete regression of Mullerian elements (12, 18, 19). Therefore, it is advisable to consider DSD in children with gonadal tumors, especially if presenting with amenorrhea, ambiguous genitalia, or other genital defects (20). Studies show that people with PAIS, with non-scrotal gonads have an increased malignancy risk; however, gonadectomy is performed based on individual cases (3). A report of a person with complete AIS having borderline serous papillary cystadenoma, also had a simple serous cystic component, hinting at the tumorigenesis and malignancy potential from the Müllerian elements (21). However, there are conflicting opinions about the timing of gonadectomy in relation to the timing of gonadal tumor malignancy in children with DSD (22). Some studies believe more complications are noted in people with DSD when diagnosed in late childhood, such as malignancy, difficulty deciding the gender, and psychosocial issues, suggesting early management of children with DSD, including prophylactic gonadectomy, can result in a better outcome (23). However post-pubertal gonadectomy is currently recommended, as gonadal neoplasm usually occurs during the post-pubertal period, and thus the people with DSD can decide for themselves, and it allows puberty to occur (24). From our study we observed 30% of children at prepuberty age had malignant tumors, while 70% were at puberty age, with 7/10 cases at stages 1-2; however, this shows there is still a possibility of malignancy in early childhood.

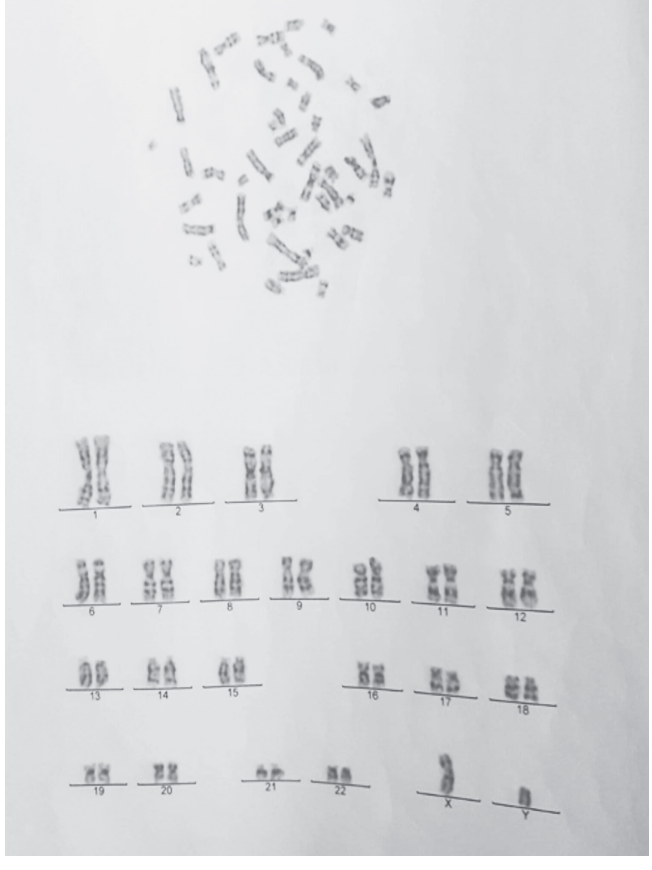

Figure 1. 46, XY karyotype result.

Gonadectomy was performed in 8/10 recent studies of 136 children with gonadal tumors and DSD from Google Scholar literature searches 2019-2023 (Table 3); although 50% of these studies supported early prophylactic gonadectomy, especially if having a high-risk malignancy DSD such as, complete or mixed gonadal dysgenesis, the opinion of three authors were unavailable. An author stated it depends on the child's will, while in the neonate study, it supports only removal of identified streak gonads without biopsy having a 50% probability of transforming into gonadoblastoma or dysgerminoma and the occurrence of an in situ tumor during biopsy (25-34).

Some studies, including two listed in Table 3 advised early investigation for DSD at pregnancy and that sex assignment be done during the neonate period, considering the complicated long process; including diagnosis, gonads present, genetic karyotype, external genitalia, the parents' wish, hormonal therapy, if surgery is required for malignancy prophylaxis or sex assignment, and fertility potential, in order to reduce

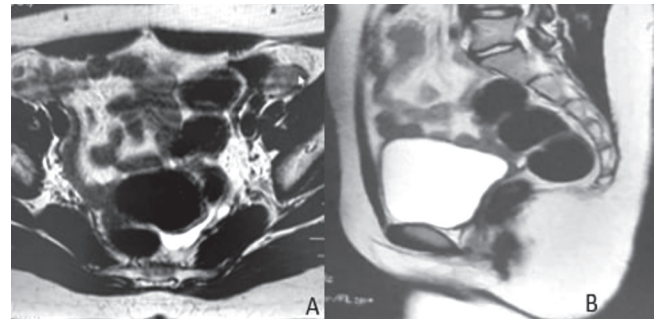

Figure 2. (a). MRI, showing the testis, T2 sagittal plane section-The uterus and vagina are not visible between the bladder and rectum. (b). T2 Axial section- The testicles are at the iliac fossa (small arrow showing the left testis).

child's psychosocial traumas before maturity and increase awareness, and knowing malignancy risk increases with age (28, 9, 32).

Dysgerminoma and cystadenoma tumors were mainly noted in our study, while the studies in Table 3 reported that gonadoblastoma and dysgerminoma mainly affected children with DSD (60%), and 50% of these studies reported the presence of other systemic malformations, however only one discussed the child's psychosocial issues (25-34). This may be due to the aims and scopes of these studies. DSD was noted in 68.8% of children during GCT diagnosis (27), While in our study 47% presented with gonadal tumor complaints, then DSD was later identified, and 6/8 of these children had malignant tumors. Furthermore, in the article of the first case in Table 3. The histopathological result of six

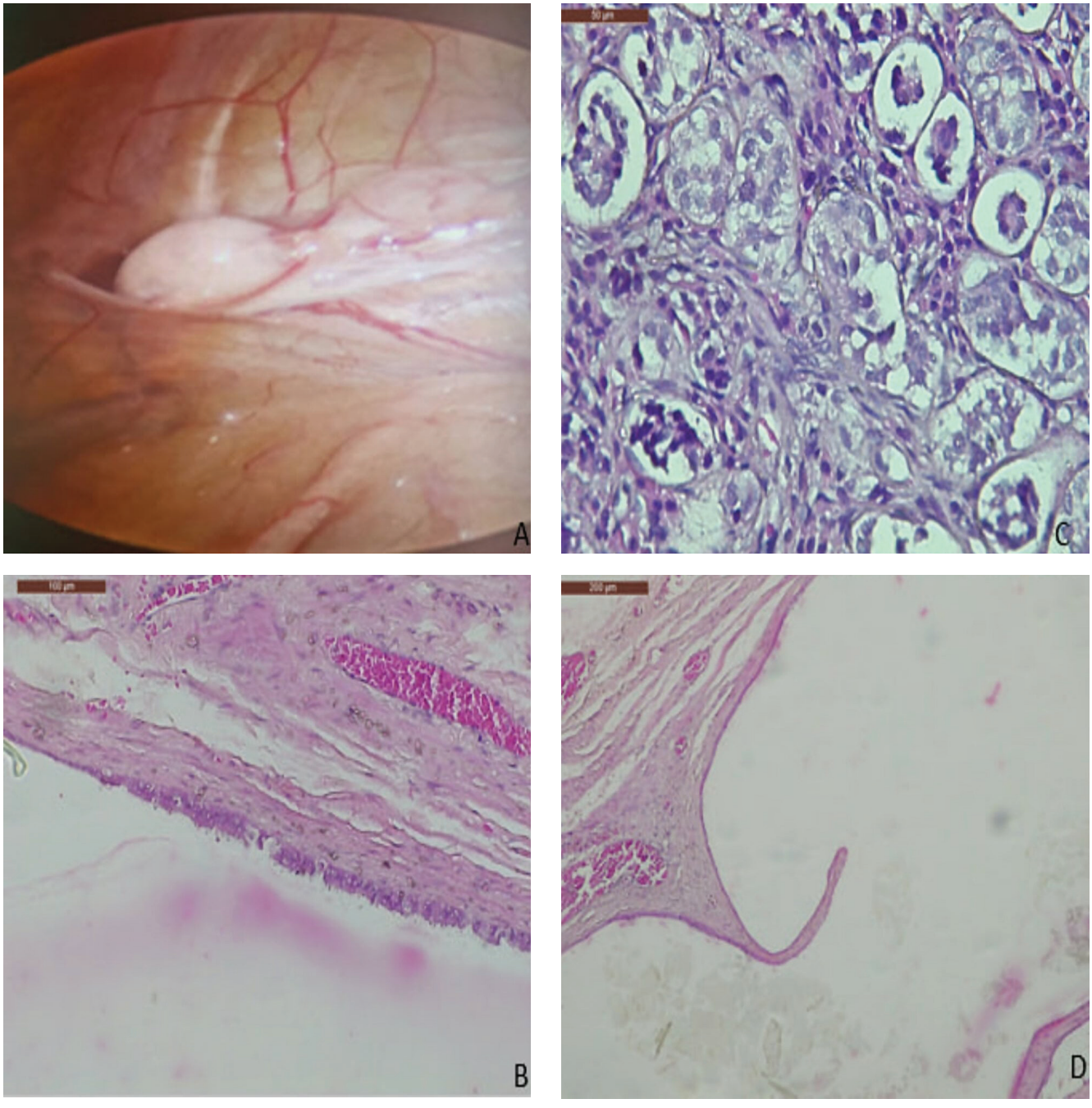

Figure 3. (a) Exploratory laparoscopy showing the right testis. Pathohistological slides, showing testicular serous papillary cystadenoma and testicular parenchyma fragment. H.E staining. Microscopic- (b) Unilocular cystic formation lined by ciliated cylindrical epithelium. The cyst wall consists of fibrous connective tissue with hyperemic dialated vascular structures, no psammoma bodies X30. (c) Testicular parenchymal fragment, partially bounded by a fibrous capsule containing crowded seminiferous tubules with lumen, fibrosis bands with lots of fibrous septations, isolated spermatocytes and Leydig cells in small groups X50. (d) Isolated papillary projection X10.

other children with DSD ≤2 years with hypospadias and inguinal hernia revealed delayed maturation of gonocytes in seminiferous tubules and primordial germ cells that expressed OCT3/4, and PLAP in immunohistochemistry study, showing that gonadal tumors in children with DSD are GCT precursor lesions (25). We noted short stature in 29.4% of our participants especially those with Turner syndrome, this is in line with the studies in Table 3, confirming that growth hormone deficiency, leading to a short stature or growth failure is usually noted in DSD with mixed gonadal dysgenesis, so a child presenting with gonadal tumor and a short stature should be evaluated for DSD (28, 34).

Apart from gonadal malformation, obesity was also noticed in most of the study participants with malignancy, this is in line with literature which recognizes obesity as a risk factor for gonadal malignancy, also we identified malignant tumors with LHD, CA125, AFP, and B-HCG, which are recognized as the standard gonadal tumor markers (35). In addition, a DSD study revealed Germ cell neoplasia in situ /GCT- and GCT-free survival at gonadal surgery decreased at age 15, and the GCT risk increased at ≥15 years old, with the 5-year overall survival worse for children with GCT ($p < 0.001$) (36); however, in our study, only a child aged 14 with dysgerminoma stage 4 died after three years. We observed lower hormone levels in children with malignancy compared to those without malignancy, showing the DSD pathogenesis determines the hormonal level; however, the tumor may have some impact on these hormones, as we saw an elevated AMH in a child with granulosa cell tumor having 5 alpha reductase deficiency, unlike the one without. A study reveals that if granulosa cell tumor size exceeds 10cm, AMH becomes undetectable or reduced, showing although the tumor present is a determining factor that affects AMH, yet, it is an unstable factor (37).

Furthermore, from the particular case we discussed, it confirms a study showing that, unlike persons with complete AIS, reduced bone mineral density is not observed in a non-gonadectomized or gonadectomized persons with PAIS adhering to hormonal therapy (38). A report showed females with hypogonadism, between 12–18 years given transdermal 17β estradiol, were feminized successfully at 18 months (39). At the end of 12 months of transdermal estradiol therapy, gradual feminization was noticed in the case of the child we discussed. The child with PAIS we discussed, attempted suicide, confirming a study suggesting the burden of the psychosocial issues faced by people with PAIS, especially when awaiting treatment (38, 10).

Limitation of the study

This is a retrospective study, with a relatively small number of children, however the DSD are rare findings. There are some missing values for hormonal levels and tumor markers that were not found or were not performed postoperatively in some cases to enable a comparison. The study did not include adults, where the risk of tumors could be increased even more, compared to pubertal children.

In conclusion, children with disorders of sex differentiation, have a higher risk of malignant gonadal tumors, if having other risk factors such as obesity and systemic malformation, also psychosocial issues were associated with pubertal age (>12 years) in this study. Diagnosis of DSD, especially at puberty raised numerous challenges that can affect achieving the best treatment management; hence early diagnosis is preferred. Communication is important among the interdisciplinary team, as well as the psycho-emotional stability of the child with DSD and their family for effective management of child. Lack of consensus recommendation for optimal time for gonadectomy in people with DSD was observed in the literature review, as this needs to be individualized for each person. Cytogenetic and molecular genetic testing can help guide the patient management.

Conflict of interest

The authors declare that they have no conflict of interest.

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