Morphometry and Histology of Gonads From 13 Children With Dysgenetic Male Pseudohermaphroditism

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● ***Background.*—Dysgenetic male pseudohermaphroditism (DMP) is a sexual differentiation disorder characterized by bilateral dysgenetic testes, persistent müllerian structures, and cryptorchidism in individuals with a 46,XY karyotype. However, the histologic criteria for the diagnosis of DMP are poorly established.**

***Objective.*—To determine gonadal histology in children with DMP.**

***Patients and Methods.*—Between 1996 and 1998, 13 patients with DMP were evaluated on our service. The clinical diagnosis of DMP was based on a 46,XY karyotype, sex ambiguity, high levels of follicle-stimulating hormone and low levels of antimüllerian hormone, a decreased testosterone response to human chorionic gonadotropin stimulation without accumulation of testosterone precursors, and the presence of müllerian structures. Molecular sequencing the HMGbox region of the SRY gene did not reveal any mutations. Biopsies were performed for 22 of 26 gonads (patient age at the time of biopsy, 16 months to 10 years). Conventional microscopy was used to evaluate mean tubular diameter, tubular fertility index, and number of Sertoli cells per tubular profile.**

***Results.*—All 26 gonads were located outside of the labioscrotal folds. Their histologic features varied from only a reduction in tubular size to features of a streak gonad. Five of the 22 gonads grossly resembled a streak gonad. The mean tubular diameter was severely reduced (>30% reduction relative to the normal tubular diameter for the patient's age) in 4 gonads, markedly reduced (10%–30%) in 11 gonads, slightly reduced (<10%) in one gonad, and normal in one gonad. The tubular fertililty index, expressed as the percentage of tubular profiles containing germ cells, was severely reduced (<30% of normal values) in 9 gonads, markedly reduced (50%–30%) in 2 gonads, and normal in 6 gonads. The number of Sertoli cells per tubular profile was elevated in 16 gonads and normal in one gonad. Thin tubules surrounded by fibrous tissue were occasionally observed.**

***Conclusion.*—The histologic findings confirmed the clinical diagnosis of DMP in every patient in the present series. However, gonadal histology was variable, and careful morphometric evaluation may be necessary to establish the diagnosis.**

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Dysgenetic male pseudohermaphroditism (DMP) is a sexual differentiation disorder diagnosed on the basis of morphologic features in patients with evidence of gonadal dysgenesis. Typically, patients have ambiguous genitalia, low testosterone levels, persistence of müllerian structures, bilateral abnormal testes, and an increased risk for gonadal neoplastic transformation.[1–4] Depending on the severity of testicular dysgenesis, the antimüllerian hormone (AMH) level may be low or undetectable in patients with abnormal testicular determination.[5,6] Typically, the gonadal histology is characterized by poorly developed seminiferous tubules surrounded by wavy ovarian stroma. However, gonadal development may range from apparently normal testicular differentiation to a streak gonad, and the criteria for the histologic diagnosis of DMP are not well established.

The most frequently observed karyotype in patients with DMP is 46,XY. However, multiple cell lines, including a monosomic X cell line, may be detected, and somatic features of Turner syndrome, such as short stature, webbed neck, *cubitus valgus*, and renal malformation, may be present.[7]

In fact, some authors consider DMP a variant of mixed gonadal dysgenesis,[4,8–9] which is defined as the coexistence of a streak gonad and a dysgenetic testis in a patient with a 45,X/46,XY karyotype.[1,8,10] In order to rule out the presence of Denys-Drash syndrome, which occurs as a consequence of mutations at the *WT1* gene on chromosome 11, patients with DMP must be examined periodically for the presence of Wilms tumor and nephropathy with progressive renal failure.[11]

The objective of this study was to better define the histologic criteria for the diagnosis of DMP by determining the morphometric and histologic features of the gonads of 13 children with a strict clinical diagnosis of this entity.

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Table 1. Clinical and Hormonal Data of 13 Children With Dysgenetic Male Pseudohermaphroditism*

Patient	Age, mo	External Genitalia†	Position of the Gonads, R/L	AMH, pmol/L	T, nmol/L‡	β-hCG, IU/L	LH, IU/L§	FSH, IU/L‖
1	7	3	I/I	118	1.4	NP	1.0	1.3
2	108	2	A/A	114	0.7	NP	0.9	5.4
3	30	2	I/I	52	<0.3	319	0.6	1.2
4	0.5	3	I/I	98	1.7¶	NP	0.8	3.9
5	10	2	I/I	114	<0.3	351	1.1	1.4
6	92	2	I/A	113	1.0	NP	0.8	5.8
7	78	2	I/−	73	<0.3	301	1.0	6.3
8	13	2	I/I	107	<0.3	196	0.5	1.0
9	20	4	I/A	11	<0.3	351	1.1	1.5
10	20	2	A/A	91	<0.3	219	1.0	1.4
11	31	3	I/I	25	<0.3	143	0.1	0.8
12	29	3	A/A	116	3.1	NP	0.3	0.7
13	44	3	I/A	71	<0.3	365	0.9	0.9

* R indicates right; L, left; AMH, antimüllerian hormone; T, testosterone; β-hCG, β-human chorionic gonadotropin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; A, abdomen; I, inguinal canal; −, not found; and NP, not performed.
† According to the classification of Quigley et al.[21]
‡ Total testosterone level after hCG stimulation test.
§ Normal values of prepubertal LH: 0.1–1.0 IU/L.
‖ Normal values of prepubertal FSH: 0.1–1.4 IU/L.
¶ Basal total testosterone level.

SUBJECTS AND METHODS

Subjects

The series consisted of 13 patients with a clinical diagnosis of DMP evaluated by the Interdisciplinary Group for the Study of Sex Determination and Differentiation at the University Hospital of the UNICAMP (Campinas, São Paulo, Brazil), between May, 1996, and May, 1998. The diagnosis of DMP was supported by the findings of ambiguous genitalia in patients with a 46,XY karyotype and bilateral cryptorchidism, low levels of testosterone and AMH, and evidence of müllerian duct derivatives. This study was approved by the Ethics Committee of the University Hospital.

The patients' mean age at first consultation was 3 years and 1 month (range, 15 days to 9 years), and the initial sex assignment was male in all cases. One patient (case 12) had a previous history of unilateral Wilms tumor and renal failure. No consanguinity was registered, and there was no family history of sex ambiguity. All individuals had hypospadias, bilateral cryptorchidism (Table 1), and evidence of müllerian duct derivatives, which was confirmed by laparoscopy.

Routine hormonal determination was performed by radioimmunoassay and included basal levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione, and dehydroepiandrosterone. Total testosterone levels were determined before and 24 hours after the last of a series of 3 daily intramuscular injections of 2000 IU of human chorionic gonadotropin (hCG) (Profasi hp, Serono Lab, São Paulo, Brazil). An increase in testosterone level of more than 4.9 nmol/L (1.4 ng/mL) was considered normal. When testosterone levels did not increase, determination of serum β-hCG was performed to confirm that it was really used. Serum AMH levels were measured by an enzyme-linked immunosorbent assay, using antibodies against human recombinant AMH,[12] in the laboratory of the Unité de Recherches sur l'Endocrinologie du Développement (INSERM), Montrouge, France. In one patient (patient 4), who was less than 6 months of age, testosterone levels were assayed only in baseline blood samples, because, at this age, testosterone values in normal children are elevated. All patients had low AMH levels as compared with the normal values published by Rey et al.[5] They also had decreased testosterone levels either at baseline or after hCG stimulation, with no accumulation of testosterone precursors. All patients had a predominance of FSH over LH, despite the fact that only 5 patients (patients 2, 4, 6, 7, and 8) had elevated gonadotropin levels (Table 1).

Genomic DNA samples from each patient and normal male and female controls were previously used as templates to amplify the HMGbox region of the SRY gene and no mutations were found after direct sequencing of polymerase chain reaction products.

Methods

The biopsies of gonads were fixed in Bouin solution, dehydrated in alcohol, and embedded in paraffin. Serial 4-μm sections from each part of the gonads were stained with hematoxylin-eosin and examined under light microscopy. The mean tubular diameter, tubular fertility index (TFI), and Sertoli cell number per tubular profile were evaluated in 50 to 100 randomly selected seminiferous tubules of each section. Our findings were compared with the normal data for age published by Nistal and Paniagua (Figure 1).[13]

The mean tubular diameter of both the longitudinal and transverse sections was measured using a calibrated vernier ocular micrometer with a 40× objective, as described by Lennox et al.[14] The reduction of diameter was classified according to Nistal and Paniagua[13] into 3 degrees of severity: slight (<10% reduction in relation to the normal diameter for the age), marked (10%–30% reduction), and severe (>30% reduction) tubular hypoplasia.

Germ cell number was evaluated by determining the TFI, which is the mean number of germ cells per tubular profile. This value was calculated by counting the number of germ cells in a single light microscope field and dividing that number by the number of tubular profiles in that given field. According to Nistal and Paniagua,[13] 3 levels of severity of germinal hypoplasia can be recognized: slight (TFI, >50%), marked (TFI, of 50%–30%) and severe (TFI, <30%).

The number of Sertoli cells per tubular profile was determined. The Leydig cell number was not evaluated because this number is low during infancy. Streak gonads were defined as those composed of an ovarian-type stroma with sclerohyaline nodules.

RESULTS

Biopsies were performed for 22 of the 26 gonads. The patients' ages at the time of biopsy ranged from 16 months to 10 years (Table 2). Biopsies were not performed for 4

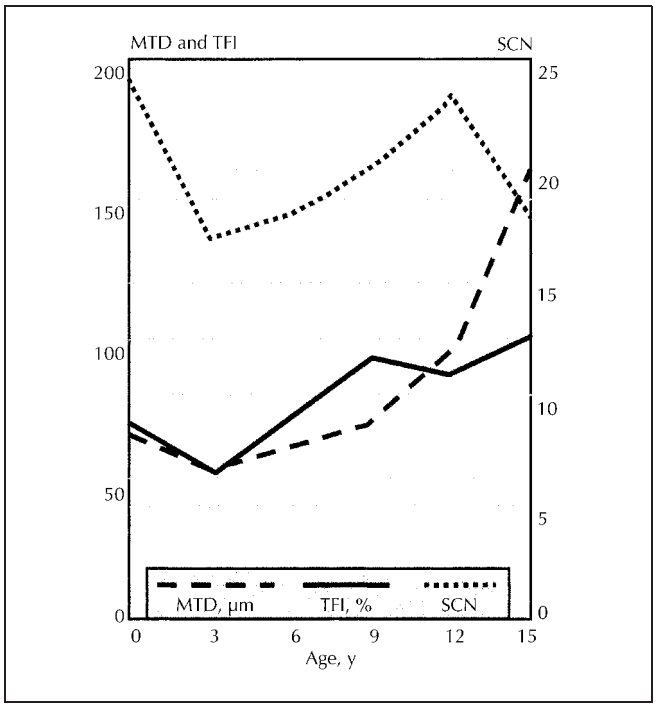

Figure 1. *Changes in the mean tubular diameter (MTD), tubular fertility index (TFI), and Sertoli cell number (SCN) profiles from birth to puberty (adapted from Nistal and Paniagua[13]).*

gonads: the left gonad of patients 1 and 7, and the right gonad of patients 2 and 4. The left gonad of patient 7 was not found, but the ipsilateral absence of müllerian duct derivatives and the presence of wolffian duct derivatives suggested a unilateral vanishing testis. In the other 3 patients, the biopsy of one of the gonads was not suitable for histologic analysis.

There were 5 streak gonads (the left gonad of patients 2, 6, 9, 10, and 13), which were all in an abdominal position.

Table 2 shows values for the mean tubular diameter, TFI, and number of Sertoli cells per tubular profile observed in the 17 remaining gonads.

Eleven gonads showed marked tubular hypoplasia, 4 showed severe tubular hypoplasia, and one showed slight tubular hypoplasia (Figures 2 through 4). The mean tubular diameter was normal in only one gonad (the right gonad of patient 13).

The TFI was severely reduced in 9 gonads, markedly reduced in 2 gonads (Figures 2 through 4), and normal in 6 gonads.

The Sertoli cell number per tubular profile was elevated in 16 of 17 gonads analyzed (Figures 2 through 4). Only the right gonad of patient 13 showed a normal number of Sertoli cells.

COMMENT

Ambiguous development of the genital ducts, urogenital sinus, and external genitalia occurs in patients with dysgenetic gonads. These patients usually present with evidence of AMH deficiency as well as androgen deficiency, and therefore have müllerian duct derivatives and ambiguous external genitalia.[8] Mutations and deletions in genes involved in the testes determination and differentiation cascade have been implicated in the etiology of DMP.[15]

Our 13 patients presented with some interesting findings: all had a male sex assignment before the first consultation, and there was a delay in the evaluation of the

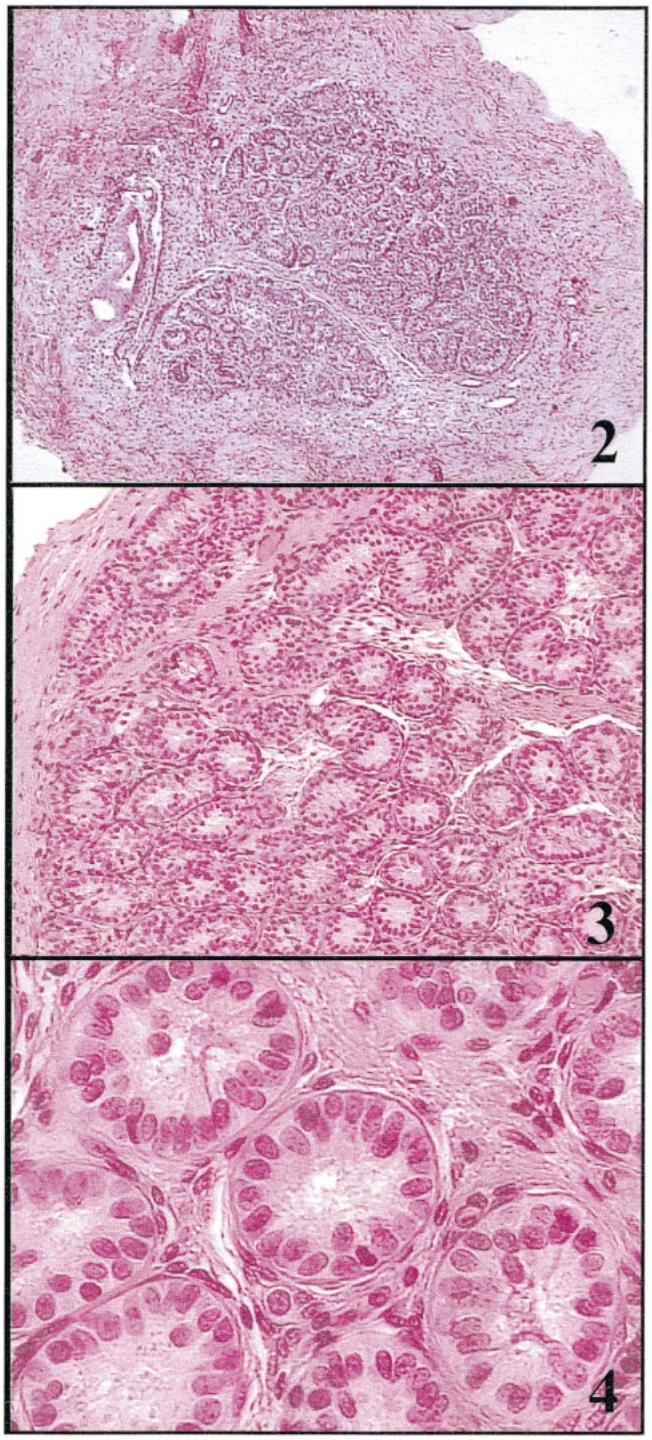

Figure 2. *Tissue from patient 4. An area of left gonad (hematoxylin-eosin, original magnification ×75).*

Figure 3. *Tissue from patient 12. An area of right gonad showing marked tubular hypoplasia, severe germinal hypoplasia, and hyperplasia of Sertoli cells (hematoxylin-eosin, original magnification ×150).*

Figure 4. *Tissue from the right gonad of patient 12 (hematoxylin-eosin, original magnification ×300).*

Table 2. Morphometric and Histologic Data of 22 Gonads From 13 Children With Dysgenetic Male Pseudohermaphroditism*

Patient	Age, mo†	No. Tubular Profiles Examined	MTD, μm	STH	TFI, %	SGH	SCN	HSC
1	11	R, 110	52.3	Marked	12	Severe	28	+
2	122	L, −	. . .	Streak	. . .	Streak	. . .	Streak
3	36	R, 69	29.1	Severe	0	Severe	19	+
		L, 104	35.8	Severe	15	Severe	22	+
4	16	L, 117	49.6	Marked	10	Severe	29	+
5	16	R, 102	46.8	Marked	78	Normal	23	+
		L, 101	49.1	Marked	97	Normal	24	+
6	108	R, 99	59.5	Slight	6	Severe	29	+
		L, −	. . .	Streak	. . .	Streak	. . .	Streak
7	84	R, 104	39.4	Severe	111	Normal	21	+
8	19	R, 126	54	Marked	68	Normal	19	+
		L, 116	52.4	Marked	166	Normal	19	+
9	26	R, 42	38.3	Severe	50	Severe	26	+
		L, −	. . .	Streak	. . .	Streak	. . .	Streak
10	27	R, 98	43.9	Marked	0	Severe	18	Normal
		L, −	. . .	Streak	. . .	Streak	. . .	Streak
11	36	R, 104	49.6	Marked	33	Marked	30	+
		L, 115	53.1	Marked	45	Marked	33	+
12	37	R, 98	49.2	Marked	0	Severe	37	+
		L, 80	43.4	Marked	10	Severe	32	+
13	51	R, 66	64.1	Normal	115	Normal	24	+
		L, −	. . .	Streak	. . .	Streak	. . .	Streak

* MTD indicates mean tubular diameter; STH, severity of tubular hypoplasia according to Nistal and Paniagua[13]; TFI, tubular fertility index; SGH, severity of germinal hypoplasia according to Nistal and Paniagua[13]; SCN, Sertoli cell number per tubular profile; HSC, hyperplasia of Sertoli cells according to Nistal and Paniagua[13]; R, right gonad; L, left gonad; −, absence of seminiferous tubules; and +, present.
† Age at which gonadal biopsy was performed.

ambiguous genitalia (mean age at the time of evaluation, 3 years and 1 month). Although all patients had a predominance of FSH over LH, only 5 of them had high FSH levels for their age. Among these 5 patients, 3 were older than 6 years, 1 was 15 days old, and 1 was 1 year old, suggesting that gonadotropins may be useful in the diagnosis of DMP at the extremes of prepubertal age. Variable degrees of masculinization of the external genitalia were noted, and the gonads were located outside of the labioscrotal folds in all patients. One patient (patient 12) had features of Denys-Drash syndrome.

The diagnostic confirmation of DMP is based on the histologic finding of variable degrees of bilateral dysgenetic testes, which range from an almost ''normal'' testis to a testis that grossly resembles a streak gonad. Although the abnormalities observed in dysgenetic testes are well defined, pathologists usually have some difficulties establishing the diagnosis of dysgenetic testes, and the characteristics of dysgenetic gonads are not routinely evaluated.

Testicular biopsy is essential for the diagnosis in some patients with ambiguous genitalia, and the evaluation of biopsies of prepubertal testes should involve assessment of the mean tubular diameter and the number of germ cells, Sertoli cells, and Leydig cells (when evaluated) per tubular profile, per unit area, per unit volume, or per testis.

Mean tubular diameter is a very good indicator of the development of the seminiferous epithelium.[13] In the prepubertal testis, this diameter depends mainly on the number of Sertoli cells, and thus indicates whether they are adequately stimulated by FSH and responsive to this stimulus. Testicular diameter varies throughout childhood; it is smallest in the fourth year of life, increases slowly until 9 years of age, and increases rapidly thereafter, up to 15 years of age (Figure 1).[13] Our patients with DMP showed a variable mean tubular diameter, from normal to severely decreased, but with a predominance of marked and severe tubular hypoplasia (15/17 gonads). Even though there are technical pitfalls in evaluating the mean tubular diameter, our data indicate the need for this evaluation in all testicular biopsies when there is clinical suspicion of gonadal dysgenesis, independent of the gonadal location.

Germ cells may be counted by several methods. The most common method is calculating the TFI, which reflects the percentage of tubular profiles containing germ cells. In neonates, 68% of tubular profiles contain at least one germ cell. From birth to 3 years, this value decreases to 50%, followed by a progressive increase to 100% at puberty (Figure 1).[13] A more complete determination of germ cell number can be obtained by calculating the total number of germ cell per testis, but this approach requires morphometric assessment of the intratubular volume and careful clinical measurement of the 3 axes of the testis.[16] The TFI was altered in 11 of the 17 testes that we evaluated. Among the 6 patients with dysgenetic testes and normal TFIs, 5 had a decreased and 1 had a normal mean tubular diameter. Therefore, our data suggest that mean tubular diameter is a better indicator of testicular dysgenesis than TFI.

Another histologic parameter evaluated was the number of Sertoli cells per tubular profile, which varies during childhood as a result of low levels of Sertoli cell proliferation between the ages of 4 and 12 years.[17] Hyperplasia of Sertoli cells is usually pronounced in patients with DMP, and it is a sign of tubular dysgenesis.[18] The number of Sertoli cells was increased in all of the gonads of our patients with DMP, but in one patient, this increase was not marked (Figure 1). This finding probably reflects the fact

that the ideal time for this evaluation is during the first year of life or at the onset of puberty.

Finally, it is important to emphasize that among the 22 evaluated gonads, only 5 grossly resembled streak gonads. It is interesting to point out that they were all found on the left side; among true hermaphrodites, there is also a predominance of ovaries and ovotestes on the left side.[19,20]

In conclusion, the histologic findings in our patients with DMP, although highly variable, confirmed the clinical diagnosis. A careful histologic and morphometric evaluation, particularly the measurement of mean tubular diameter, may help establish and improve the diagnosis of DMP.

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