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BRIEF REPORT

Testicular Adrenal Rest Tumors in Adult Males with Congenital Adrenal Hyperplasia: Evaluation of Pituitary-Gonadal Function before and after Successful Testis-Sparing Surgery in Eight Patients

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Context: In male patients with congenital adrenal hyperplasia (CAH), testicular adrenal rest tumors (TART) are frequently present. These tumors can interfere with testicular function. Intensifying glucocorticoid therapy does not always lead to tumor regression and improvement of testicular function. Recently, testis-sparing surgery was introduced for treatment of TART.

Objective: The aim of this study was to evaluate tumor volume, symptoms, and pituitary-gonadal function in male patients with CAH caused by 21-hydroxylase deficiency and bilateral TART before and after testis-sparing surgery.

Setting: This study was conducted at Radboud University Nijmegen Medical Centre in The Netherlands.

Patients: Eight adult male CAH patients with bilateral TART and infertility were included.

Interventions: Evaluation of testicular magnetic resonance imaging, symptoms, fasting serum concentrations of ACTH, LH, FSH, inhibin B, 17-OH progesterone, androstenedione, testosterone, and estrone, and semen analysis (six of eight patients) was performed before and 6 and 22 months after testis-sparing surgery.

Main Outcome Measures: The main outcome measures were absence of residual tumor and improvement of symptoms and pituitary-gonadal function.

Results: Residual tumors were not found on any of the patients' magnetic resonance imaging after surgery. Two patients reported testicular pain and discomfort that disappeared after surgery. Parameters of pituitary-gonadal function did not improve after surgery: semen analysis showed azoospermia (five patients) or oligospermia (one patient) without improvement, and all patients had persistently low inhibin B concentrations.

Conclusion: Testis-sparing surgery did not improve pituitary-gonadal function despite successful removal of the tumors. Further studies are needed to investigate whether surgery at an earlier stage in the natural history of TART can prevent permanent testicular damage. (*J Clin Endocrinol Metab* 92: 612–615, 2007)

I N ADULT MALE patients with congenital adrenal hyperplasia (CAH), testicular adrenal rest tumors (TART) are frequently present with a reported incidence of 50–95% (1, 2). Because of their location in the mediastinum testis, these tumors can lead to obstruction of seminiferous tubules. In addition to these mechanical effects of the tumor, steroids produced by the tumor may reach the circulation interfering with the secretion of FSH and LH by the pituitary and they may also be toxic to testicular tissue in a paracrine manner, thereby contributing to testicular dysfunction (3–5).

Treatment with high doses of glucocorticoids may lead to

suppression of ACTH secretion and reduction of tumor size (6–9). However, high doses of glucocorticoids do not always restore testicular function and may have several side effects (10).

Because of the benign character of the tumors, testis-sparing surgery has been proposed for the treatment of TART. Walker *et al.* (11) performed testis-sparing surgery in three CAH patients. Postoperatively, there was good vascular flow and no recurrence of the tumor. Tiryaki *et al.* (12) reported two CAH patients with steroid-unresponsive testicular tumors who were also treated by testis-sparing surgery. In both studies no information about pituitary-gonadal function before and after surgery was reported.

We treated eight adult infertile CAH patients with bilateral TART with testis-sparing surgery. The aim of our study was to evaluate whether testis-sparing enucleation of the tumor can improve pituitary-gonadal function. Here we describe the results of the clinical, biochemical, radiological, and histological evaluation of the patients before and 6 and 22 months after the operation.

Patients and Methods

Patients and surgical procedure

Eight male patients with CAH caused by 21-hydroxylase deficiency were selected for operation. Written informed consent was obtained from all patients. All patients had bilateral TART. Five patients had palpable masses (patients 1–4 and 6). Two patients reported pain and discomfort. Five patients (patients 1, 2, and 4–6) had been treated with high doses of glucocorticoids in the past to reduce tumor size without success. The indications for operation are listed in Table 1. The age of the patients was 30 ± 8.9 yr (mean ± SD; range, 23–51 yr). Height SD score was −2.0 ± 1.0 (range, −3.5–−0.2) and body mass index (BMI) was 28.1 ± 4.4 kg/m^2 (range, 23.7–38.2). Seven patients were white and one patient (patient 8) was of West Indian ethnicity.

Testicular tumor enucleation took place after general or locoregional anesthesia. The testis, including its tunica vaginalis, was luxated through an inguinal incision and the tunica vaginalis was opened. The testis was incised at the margo anterior testicularis through the testicular tissue until the margin of the tumor was reached. Then, a careful blunt dissection of the tumor was undertaken. Finally, the tunica albuginea and the tunica vaginalis were closed and the testis was repositioned in the scrotum.

Biochemical analysis

Biochemical analysis was performed in all patients before and after operation. Patient 1 underwent operation without complete preoperative hormonal and radiological evaluation. Venous blood was collected from an antecubal vein at 0900 h after overnight fasting and before taking the morning medication to measure serum levels of 17-OH progesterone (17OHP), androstenedione (A), testosterone, estrone, ACTH, LH, FSH, and inhibin B. The next day at 0900 h, after overnight fasting, blood was collected after taking 8 mg dexamethasone orally at 2300 h the evening before to measure serum levels of 17OHP, A, testosterone, estrone, and ACTH. The same investigations were performed 6 months after operation in all patients. Twenty-two months after operation, again venous blood was collected after overnight fasting and before taking the morning medication to measure the same parameters as described. Glucocorticoid doses were not changed until completion of the evaluation 6 months after the operation.

Hormone assays

Serum testosterone and 17OHP were assessed by ^{3}H-RIA after prepurification by means of paper chromatography of ether extracts of the samples, as described previously (13, 14). Serum A concentrations in serum and saliva were measured as described earlier (14). Serum estrone was measured by RIA after extraction and Sephadex LH-20 chromatography. The within- and between-assay coefficients of variation were 4.8% and 7.5%, respectively. ACTH was measured by a two-step immunoradiometric assay (Dynotest BRAHMS, Berlin, Germany). Serum FSH and LH were determined with a Fluorescence Immuno Enzymatic Assay (Abbott Diagnostics, Hoofddorp, The Netherlands) using a Random Access Analyser (Type AxSYM; Abbott). Dimeric inhibin B was quantified using an ELISA (Oxford Bio-Innovation Ltd., Oxford, UK).

Semen analysis

In six of the eight patients, semen analysis was performed after more than 2 d of sexual abstinence before and 6 and 22 months after operation (15). One patient (patient 5) refused semen analysis. Another patient (patient 6) was sterilized in the past.

Radiological evaluation

All patients underwent testicular magnetic resonance imaging (MRI) before and 6 and 22 months after surgery. All MR studies were performed on a 1.5-T scanner (Magnetom Sonata, Symphony or Avanto; Siemens, Erlangen, Germany), using a body phased-array coil.

Histopathology

All removed tumor tissue was investigated macroscopically and microscopically. Tumors and testis biopsies were fixed in 10% buffered formalin. Tissue sections of 5 μm were cut and stained with hematoxylin and eosin and with Von Gieson elastin stain. Testis biopsy specimens were scored according to Johnsen with a score varying from 0 to 10. A Johnsen score of more than 8 is associated with fertility (16).

Results

Radiological evaluation

No apparent residual tumor was seen in any patient on postoperative images. The measured volume of the testicular tumor (mean, 9.6 ml; range, 0.5–29.6) showed a good correlation with the tumor weight (R^2 = 0.98). Testicular volumes decreased after surgery in all patients (range, −8 to −87%).

TABLE 1. Age, phenotype, mutation analysis, height corrected for target height, BMI, and daily glucocorticoid and mineralocorticoid therapy at time of operation, and operation indication in 8 male CAH patients with bilateral testicular adrenal rest tumors

Patient	Age (yr)	Phenotype[a]	Allele 1[b]	Allele 2[b]	Height (SDS)[c]	HSDS-THSDS[c]	BMI (kg/m^2)	Daily glucocorticoid therapy (mg/m^2)[d]	Daily mineralocorticoid therapy (μg)[e]	Operation indication[f]
1	24	SW	Deletion/conversion	Deletion/conversion	−2.6	−2.8	27.4	32.2 (HC 20–20-20 mg)	400	1,2
2	29	SW	Deletion/conversion	Deletion /conversion	−2.1	−0.7	25.7	16.0 (HC 20–10 mg)	125	1,2
3	23	SW	IVS2–13A/C>G	IVS2–13A/C>G	−1.4	−0.7	25.6	8.2 (HC 8–4 mg, DXM 0.1 mg)	62.5	2
4	32	SW	IVS2–13A/C>G	IVS2–13A/C>G	−1.4	n.a.	28.3	16.9 (HC 25–10 mg)	100	2,3
5	26	SW	IVS2–13A/C>G	IVS2–13A/C>G	−1.9	−1.0	38.2	10.8 (HC 10–5-10 mg)	62.5	1,4
6	51	SV	I172N	Deletion /conversion	−3.1	−2.95	29.0	16.2 (HC 20–10 mg)	—	5
7	31	SW	Deletion/conversion	Deletion/conversion	−0.2	−1.3	27.0	30.1 (HC 25–40 mg)	125	2
8	26	SW	IVS2–13A/C>G	IVS2–13A/C>G	−3.5	−2.9	23.7	12.1 (DXM 0.5 mg)	62.5	2,3

DXM, Dexamethasone; HC, hydrocortisone; n.a., not available; SDS, SD score.
[a] SW, Classic salt wasting CAH; SV, classic simple virilizing CAH.
[b] Nucleotides are numbered according to Higashi's functional CYP21 sequence (18).
[c] Height is expressed as SDS and corrected for target height SDS (HSDS-THSDS).
[d] Doses of dexamethasone were converted to hydrocortisone equivalents (1 mg dexamethasone = 40 mg hydrocortisone).
[e] Mineralocorticoid medication (9-α-fluorohydrocortisone acetate) was taken in one to three doses.
[f] 1, Poor hormonal control; 2, infertility; 3, pain/discomfort; 4, hypogonadotropic hypogonadism; 5, hypergonadotropic hypogonadism.

Biochemical analysis (Table 2)

Before operation, inhibin B levels were significantly decreased in all patients without any correlation with FSH levels. In four patients (patients 1, 2, 4, and 5) estrone levels were markedly increased with a strong correlation with A levels ($R^2 = 0.9$; $P < 0.02$). In three of these patients (patients 1, 2, and 5) we found suppressed LH and FSH levels suggesting suppression of the hypothalamic-pituitary-gonadal axis caused by high-serum estrone levels induced by aromatization of A. Despite this we found serum testosterone levels that were not decreased or were even in the high normal range (patient 1). Additionally, testosterone levels decreased after overnight high-dose dexamethasone in these three patients (data not shown), indicating that testosterone in blood was mainly derived from conversion of adrenal androgens. Two patients (patients 6 and 7) showed elevated levels of LH and FSH, and in one patient (patient 6) with a low testosterone concentration. In two patients (patients 3 and 8) LH and FSH and testosterone levels were within the normal range. In these four patients (patients 3, 6, 7, and 8) estrone levels were also within the normal range.

After operation inhibin B levels remained low in all patients with again no significant correlation with FSH levels. The patients showed a variable increase in FSH and LH levels except in 1 patient (patient 1) who had persistently low LH and FSH levels. Two patients showed a significant decrease in testosterone levels, suggesting additional testicular damage due to surgery.

Semen analysis

Before operation azoospermia was found in five patients and oligozoospermia was found in one patient. After operation there was no improvement of sperm quality.

Histopathology

Weight of the tumors in the left testes was 9.3 ± 9.9 g (mean ± SD; range, 1.3–27.4) and in the right testes 8.7 ± 9.1 g (range, 0.45–22.9). Macroscopically, all tumors were firm and multilobular with a yellow to tan color on cut surface and narrow bands of fibrous tissue. Microscopically, the tumors consisted of sheets or confluent cords of large polygonal cells with abundant eosinophilic cytoplasm, separated by dense fibrous tissue strands located in the hilar area of the testis with compression of the rete testis.

The testicular biopsy specimens showed decreased spermatogenesis with reduced Johnsen scores (range, 1.0–7.6). In all patients focal interstitial fibrosis and peritubular fibrosis was present and in four of the seven patients focal tubular hyalinization was prominent, being most pronounced in patient 6.

Discussion

Our study is the first to our knowledge to provide a complete evaluation of pituitary-gonadal function before and after testis-sparing surgery in CAH patients with TART. All surgical procedures were without complications. Six and 22 months after surgery, MRI examination of the testes showed no evidence of residual or recurrent testicular tumor. Symp-

TABLE 2. Testicular volumes measured by MRI and parameters of pituitary-gonadal axis function and levels of 17OH progesterone and androstenedione before and 6 and 22 months after testis-sparing surgery in eight male CAH patients with bilateral testicular adrenal rest tumors

Patient	Testicular volume (ml)[a]			Serum FSH (U/liter)[b]			Serum LH (U/liter)[c]			Serum testosterone (nmol/liter)[d]			Serum inhibin B (ng/liter)[e]			Serum 17OHP (nmol/liter)[f]			Serum A (nmol/liter)[g]			Serum estrone (pmol/liter)[h]		
	Pre	Post 6	22	Pre	Post 6	22	Pre	Post 6	22	Pre	Post 6	22	Pre	Post 6	22	Pre	Post 6	22	Pre	Post 6	22	Pre	Post 6	22
1 L	nd	nd	1.7	<0.2	<0.2	0.7	<0.2	0.2	0.1	37.0	23.0	3.6	nd	nd	0	720	160	340	100	74	131	2700	4100	790
1 R	nd	nd	1.9																					
2 L	14.2	6.0*	4.9	0.6	12.3	0.7	<0.2	7.1	0.2	14.0	10.0	13.0	76	25	61	480	410	834	86	60	98	1100	1200	1900
2 R	17.6	9.9	9.5																					
3 L	8.5	2.9	2.2	8.6	79.2	53.0	5.2	62.6	43.6	17.0	9.5	11.0	65	16	23	26	18	62	1.5	0.74	4.8	140	160	300
3 R	8.1	3.4	3.8																					
4 L	8.4	6.4*	6.4	15.9	24.0	24.2	2.9	20.8	17.4	13.0	15.0	12.9	47	19	15	367	470	840	14	23	53.2	650	810	1380
4 R	9.7	6.5*	7.3																					
5 L	10.9	5.9	8.0	<0.2	4.9	30.0	<0.2	0.6	10.0	9.8	12.0	11.0	80	17	31	865	630	390	50	52	25	1400	1110	530
5 R	11.1	3.5	5.3																					
6 L	6.9	6.3	5.5	55.2	63.3	17.2	44.9	37.2	5.2	7.1	1.3	9.8[i]	10	4	<10	5.1	1.7	2.0	0.9	0.08	2.8	210	230	233
R	8.4	5.7	4.5																					
7 L	4.5	3.6	4.1	39.3	42.2	44.5	12.3	10.2	19.4	18.0	18.0	10.0	9	17	15	4.3	2.7	12.0	1.2	1.1	0.96	200	160	130
7 R	7.0	6.2	5.6																					
8 L	7.6	1.4	1.0	6.3	40.0	<0.2	5.6	28.2	<0.2	20.0	13.0	34.1[i]	5	18	25	10	3.0	195	2.2	0.68	12.6	230	130	284
8 R	6.6	4.8	3.4																					

L, Left testes; R, right testes; nd, not determined; Post, 6 and 22 months after operation; Pre, before operation.
[a] Testicular volume determined by MRI (* testis showing irregular-shaped hyposignal area adjacent to the mediastinum testis after surgery).
Normal values of our laboratory: [b] FSH, 1.5–11 U/liter; [c] LH, 1.4–8.5 U/liter; [d] testosterone, 11–45 nmol/liter; [e] inhibin B, 150–400 ng/liter; [f] 17OHP, 2.0–10.8 nmol/liter (morning); [g] A, 1.4–9.7 nmol/liter (morning); [h] estrone, 65–220 pmol/liter; [i] with suppletion of testosterone.

toms of testicular pain and discomfort as reported in two patients disappeared after surgery.

Semen analysis did not improve after surgery with persistently low inhibin B levels in all patients reflecting persistent Sertoli cell dysfunction. As seen in patients 1, 2, and 5, Sertoli cell dysfunction can be masked by simultaneous suppression of FSH secretion caused by high-serum estrone levels induced by aromatization of adrenal A in these patients. Therefore, inhibin B is a more accurate marker for Sertoli cell function than FSH in CAH patients.

The absence of positive effects on testicular function after operation despite complete removal of the tumors strongly suggests preexisting irreversible testicular damage in our patients. Indeed, peritubular fibrosis and tubular hyalinization was seen in testes biopsy specimen taken during surgery, which confirms irreversible damage of the testes probably caused by longstanding mechanical obstruction in all patients. It is clear that at this stage surgery can no longer help to restore testicular function.

TART may produce steroids that can contribute to elevated levels of A and 17OHP. Therefore, removal of the testicular tumors may lead to a decrease in the levels of A and 17OHP. However, in our group 17OHP and A levels did not change significantly after surgery. These observations suggest that surgical treatment is not helpful in improving hormonal control.

Interestingly, all but one of our patients had a homozygous deletion/conversion genotype or a homozygous IVS2–13A/C>G genotype. In an earlier study we showed that in patients who were homozygous or heterozygous for the deletion/conversion mutation tumor size was significantly larger than in patients with other mutations (17). The present study suggests that the IVS2–13A/C>G mutation may also be a risk factor for the development of testicular tumors.

In summary, testis-sparing surgery in CAH patients is a feasible treatment for TART in CAH patients. Symptoms of testicular pain and discomfort disappeared after surgery. However, 22 months after surgery no improvement in testicular function was seen. Further studies should investigate whether, at an earlier stage in the natural history of the TART, testis-sparing surgery might be advantageous.

Acknowledgments

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