

Prevalence of Testicular Adrenal Rest Tumor and Factors Associated with Its Development in Congenital Adrenal Hyperplasia

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Keywords

Congenital adrenal hyperplasia/complications · Adrenal rest tumor · Testicular neoplasms · Steroid 21-hydroxylase · Testis

Abstract

Background: Testicular adrenal rest tumors (TART) can cause infertility in congenital adrenal hyperplasia (CAH) males. **Aims:** To determine TART prevalence in patients with CAH due to 21-hydroxylase deficiency (21-OHD) and evaluate possible factors associated with its development. **Methods:** This is a descriptive and analytical cross-sectional study evaluating males with the classical form of 21-OHD through testicular ultrasonography and serum inhibin B dosages. Data on prescribed glucocorticoid dose and serum levels of 17-hydroxyprogesterone (17-OHP), androstenedione (Andro), ACTH, renin, and LH were obtained from medical records. **Results:** Thirty-eight males were evaluated. The mean age on ultrasonography was 15.2 ± 6.7 (3–27) years. Nine patients (23.7%) had TART, 4 of them were prepubertal and the

youngest was 5 years old. No association was found between TART and 21-OHD phenotype, glucocorticoid dose, or 17-OHP, ACTH, LH, renin, and inhibin B levels measured in the 6 preceding years. However, 50% of the patients who presented increased Andro 2 years prior to the evaluation had TART ($p = 0.018$, OR = 8.00 [95% CI: 1.42–44.92]), whereas in the normal Andro group only 16.7% had tumors. **Conclusion:** This study showed that TART can occur in prepubertal patients and that disease control could be a factor associated with its development. Therefore, we suggest investigating TART development early in childhood, mainly in poorly controlled 21-OHD patients.

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Introduction

Testicular adrenal rest tumors (TART) are benign lesions, often found in patients with congenital adrenal hyperplasia (CAH), and can be a cause of infertility [1, 2]. They were first reported in adults [1]; however, its occur-

rence in children was also demonstrated [3–8]. Although there is no description of malignancy for this type of tumors, they can cause infertility due to the compression of seminiferous tubules [1]. Their etiology is not well established yet. However, it has been suggested that they derive from ectopic adrenal cells remaining in the testes from the embryonic period [9–11].

Inadequate control of CAH has been shown as the main predictor for TART development, since they are ACTH responsive [12], have ACTH receptors [13, 14], and intensifying treatment can lead to the regression of lesions [15, 16]. Conversely, some patients that are inadequately treated do not develop TART. The most likely explanation is that only some of them have ectopic adrenal rest cells in testes, which would be necessary for tumor development [2].

Some authors found an increased TART prevalence in patients that were at pubertal or postpubertal periods, which can be explained by the presence of LH receptors in these tumors that would be stimulated by higher LH concentrations after puberty onset [2, 8]. Other authors have reported that TART development could be related to the degree of 21-hydroxylase enzyme impairment, therefore it would depend on the *CYP21A2* genotype [17].

The literature reports TART prevalences varying from 0 to 94%, depending on the selection of patients and the method for detection of lesions [1]. Due to their central location in the testes, only those over 2 cm would be detected upon palpation; therefore, their diagnosis requires investigation using indirect methods. Magnetic resonance imaging (MRI) and ultrasonography are equally efficient in detecting TART, therefore ultrasonography should be the choice in this case as it is a faster and lower-cost method compared to MRI [1, 18].

The function of seminiferous tubules can be evaluated by serum inhibin B, which is produced by Sertoli cells. It exerts negative feedback to FSH secretion and is the best endocrine marker of spermatogenesis in subfertile men [19]. It can also be used in the evaluation of gonadal function in prepubertal children [1, 19].

The objectives of this study were to determine TART prevalence in 21-hydroxylase deficiency (21-OHD) male patients through testicular ultrasonography and to evaluate factors associated with its development, such as the clinical form of the disease, *CYP21A2* genotype, laboratory disease control, and presence of puberty.

Patients and Methods

This is a descriptive and analytical cross-sectional study that evaluated all male patients with the classical form of 21-OHD, assisted at the Outpatient Pediatric Endocrinology Clinic of University of Campinas Clinical Hospital, Brazil.

The patients were classified as salt wasting (SW) or simple virilizing (SV) according to their clinical presentation. The classic 21-OHD diagnosis was confirmed by *CYP21A2* analysis [20, 21].

Treatment of patients consisted of the use of hydrocortisone acetate, ideally in 3 equal oral doses daily until they attained final height (FH). After puberty, most patients switched to treatment with longer-acting glucocorticoids. Fludrocortisone was administered in individual doses to SW patients and to those SV patients that presented high serum renin levels.

TART investigation was performed by scrotal ultrasonography obtained in a Toshiba Xario apparatus with high-frequency linear transducers (5–12 MHz). We performed longitudinal and transverse scans calculating the largest axial diameter of the tumors.

At the same time, serum inhibin B levels (pg/mL) were evaluated by ELISA solid-phase/plate-colorimetry using a DSL kit.

Dosages of serum 17-hydroxyprogesterone (17-OHP) (ng/mL; RIA, DSL, or Beckman Coulter-Immunotech kits), androstenedione (Andro) (ng/mL; Chemiluminescence, IMMULITE kit), ACTH (pg/mL; Immunoassay/Chemiluminescence, IMMULITE kit), renin (pg/mL; RIA, Beckman Coulter kit), and LH (UI/L; Immunoassay/Electrochemiluminescence, 2010 Elecsys kit, Roche) were obtained from medical records. Hormonal evaluations were performed at the Laboratory of Clinical Pathology, University of Campinas Clinical Hospital (HC-UNICAMP), according to service routine, ideally in the morning.

Other collected data were: birth date, age at diagnosis and at first visit to our center, beginning of treatment, *CYP21A2* mutations, and bone age (Tanner and Whitehouse method TW2 – 20 bones) at the nearest testicular ultrasonography. The difference between bone age and chronological age (Δ BA – CA) was calculated in those who had not reached FH.

According to Wedell et al.'s [22] criteria, the patients were classified into 4 genotype groups: N (null), which corresponds to the complete impairment of enzymatic activity; A, with an important but not complete commitment of enzyme activity; B, with mild impairment of enzyme activity, rarely associated with salt loss; and C, genotype usually associated with late-onset CAH.

Patients were classified into 2 groups based on puberty: the prepubertal group and the group at either puberty or postpuberty. Puberty onset was considered as Tanner P2G2 stage and a testicular volume of 4 mL or more, with progression of characters and/or increasing LH levels, and complete puberty when G5P5 stage was attained.

Adjustments of glucocorticoid doses were made based on Andro levels, clinical parameters including growth velocity, and bone age evolution.

In order to evaluate possible factors associated with TART development, all patients' consultations during the 6 years preceding the testicular ultrasonography were selected. This 6-year period was divided into three 2-year intervals, considering as time zero the nearest ultrasonography consultation. Accordingly, the intervals (–2/0), (–4/–2), and (–6/–4) were set up corresponding to the last 2 years, to the period between 4 and 2 years, and to that between 6 and 4 years prior to ultrasonography, respectively. Some

Table 1. Clinical, laboratory, and ultrasonography characteristics of the group of patients with CAH-21 and TART

	Patients' identification								
	1	2	3	4	5	6	7	8	9
Genotype	N	B	A	A	A	A	A	N	N
Clinical form	SW	SV	SW	SW	SW	SW	SW	SW	SW
Age at treatment onset, months	0.9	40.8	0.5	13.9	6.5	0.8	1.9	2.2	2.8
Age at ultrasonography, years	27.1	23.0	16.8	15.8	15.6	10.3	8.9	7.5	5.5
Δ BA-CA at ultrasonography	–	–	–	–	1.4	3.6	–0.5	2.3	3.6
Height SDS ¹	–1.33	–0.12	–1.14	–0.28	–0.92	1.58	–1.12	–0.09	1.20
Mean TH SDS	–1.04	0.02	–0.20	0.16	–0.10	–0.01	–1.33	0.27	0.47
(FH – TH) SDS	–0.29	–0.14	–1.24	–1.04	–	–	–	–	–
BMI SDS ¹	3.59	–1.82	0.41	0.81	0.64	2.28	2.33	1.00	2.17
Puberty ¹	yes	yes	yes	yes	yes	no	no	no	no
Average glucocorticoid dose, mg/m ² BS/day	8.98	8.05	16.72	15.24	13.34	15.49	12.13	21.19	19.67
Average 17-OHP level (–2/0), ng/mL	1.6	20.8	25.5	70.9	41.0	3.9	2.4	4.0	142.8
Average Andro level (–2/0), ng/mL	0.9	5.5	5.8	6.9	3.6	0.3	0.3	0.3	3.4
Average ACTH level (–2/0), pg/mL	10.0	68.0	67.7	153.5	350.0	51.2	60.6	15.9	68.5
17-OHP $\uparrow$ (–6/0), %	25.0	62.5	73.7	65.0	81.8	18.2	4.8	52.6	91.2
Serum inhibin B, pg/mL	53.0	111.0	297.0	189.0	196.0	84.0	136.0	88.0	150.0
Node diameter, cm	0.6	0.8	0.8	2.9	0.6	0.6	1.0	0.2	0.5

Δ BA – CA, difference between bone age and chronological age; SDS, standard deviation score; TH, target height; (FH – TH) SDS, difference between final height and target height SDS; BMI, body mass index; BS, body surface; 17-OHP, 17-hydroxyprogesterone; Andro, androstenedione; 17OHP $\uparrow$ (–6/0), percentage of 17-OHP altered in the last 6 years before ultrasonography; node diameter, larger diameter of the greatest testicular node. Δ BA – CA was calculated only for those patients who did not reach FH (cases 5–9); (FH – TH) SDS was calculated only for those patients who reached FH (cases 1–4).

¹ Measured at the nearest ultrasonography consultation.

parameters were also studied in the total 6-year period (–6/0) preceding testicular ultrasonography. Three patients had total monitoring periods below 6 years.

Relative to the ultrasonography evaluation, consultation 0 (C[0]), consultation –2 (C[–2]), and consultation –4 (C[–4]) corresponded to the time of the procedure, 2 years before the procedure, and 4 years before the procedure, respectively. Standard deviations scores (SDS) for height [23], body mass index (BMI) [24], and average as well as lower and upper limits of parental target height (TH) [25] were determined. The difference between the FH and TH (FH SDS – TH SDS) was calculated in patients with complete growth.

The laboratory disease control evaluation was based on dosages of serum 17-OHP, Andro, ACTH, and renin. The medians of each parameter were used in the evaluation of their biannual levels.

According to the reference values, defined for each method and laboratory kit, the patients were classified into 2 groups: *Normal group for the method (GM – nl)* when the biannual median was within the normal range for age/puberty stage and *Increased group for the method (GM – increased)* if the median was above the normal range.

Regarding the 17-OHP levels considered by the literature as an acceptable disease control [26, 27], the patients were divided into 2 other groups: *Group 17-OHP <10 ng/mL (G <10)* and *Group 17-OHP $\geq$ 10 ng/mL (G $\geq$ 10)* according to the median of this parameter in the considered period.

The percentage of high levels of 17-OHP ($\geq$ 10 ng/mL) and Andro (above the upper normal limit of the method) related to the

total evaluations performed within 6 years and in the last 2 years (–2/0) of monitoring was also calculated.

Mean prescribed doses of glucocorticoids were calculated for each period and converted into hydrocortisone equivalents as following: 0.75 mg dexamethasone = 5 mg prednisone = 20 mg hydrocortisone [17, 27, 28].

Data were analyzed with SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA) and Epi-Info (WHO, Geneva, Switzerland).

The relative and absolute frequencies of qualitative variables are presented in the tables. The mean, standard deviation, minimum, maximum, and median values of the quantitative variables were determined, as well as the prevalence and confidence interval (CI) of 95% of lesion presence.

The means of 2 independent groups were compared using Student's *t* test when the variables were adjusted to the normal distribution. A log transformation of skewed distributed data was made to analyse them with the Student's *t* test; otherwise, the Mann-Whitney test was used.

The χ^2 test or, when indicated, Fisher's exact test for a 2 $\times$ 2 table or the Fisher-Freeman-Halton exact test for larger tables were used to compare proportions.

The odds ratio (OR) was calculated by univariate logistic regression. The adjusted OR values were obtained by multivariate logistic regression, forward stepwise method (Wald). In all cases, the significance level of 5% ($\alpha = 0.05$) was adopted.

This study was approved by the Ethics Committee of UNICAMP Medical Sciences School (No. 325/2008).

Table 2. Comparison of the clinical characteristics of the patient groups with and without TART

Clinical features	TART		<i>p</i>
	present (<i>n</i> = 9)	absent (<i>n</i> = 29)	
SW/SV, <i>n</i>	8/1	16/13	0.115 ^a
Genotype null/A/B/C, <i>n</i>	3/5/1/0	10/9/9/1	0.536 ^b
Age at ultrasonography, years	14.5 [5.5 to 27.3]	15.4 [3.6 to 27.6]	0.737 ^c
Treatment onset, years	0.2 [0.04 to 3.4]	0.3 [0.03 to 7.5]	0.192 ^d
Duration of treatment, years	14.7 [5.3 to 27.1]	13.0 [1.0 to 23.8]	0.823 ^d
Follow-up, years	13.3 [4.46 to 25.4]	12.9 [1.1 to 23.9]	0.959 ^d
BMI SDS at ultrasonography	1.0 [−1.8 to 3.59]	0.5 [−2.4 to 4.3]	0.083 ^d
Height SDS at ultrasonography	−0.3 [−1.3 to 1.6]	−0.9 [−3.6 to 3.2]	0.250 ^d
Final height SDS	−1.1 [−1.4 to −0.1] (<i>n</i> = 4)	−1.6 [−3.8 to 0.3] (<i>n</i> = 17)	0.089 ^d
Prepubertal/pubertal, <i>n</i>	4/5	7/22	0.401 ^b
Glucocorticoids (HC/P/D/HC+D) at ultrasonography	6/1/2/0	15/3/7/4	–
Equivalent dose HC (−6/−4), mg/m ² BS/day	14.0 [6.2 to 20.8] (<i>n</i> = 9)	13.2 [5.8 to 19.3] (<i>n</i> = 26)	0.588 ^c
Equivalent dose HC (−4/−2), mg/m ² BS/day	13.2 [5.7 to 17.9] (<i>n</i> = 9)	12.8 [6.8 to 19.9] (<i>n</i> = 27)	0.791 ^c
Equivalent dose HC (−2/0), mg/m ² BS/day	14.5 [8.0 to 21.2]	12.7 [6.4 to 20.9]	0.251 ^c

Values are given as median [minimum and maximum], unless otherwise indicated. SDS, standard deviation score; SW, salt wasting; SV, simple virilizing; BMI, body mass index; HC, hydrocortisone; P, prednisone; D, dexamethasone; BS, body surface.

^a Bilateral probability of Fisher's exact test. ^b Bilateral probability of the Fisher-Freeman-Halton exact test. ^c Bilateral probability of Student's *t* test. ^d Mann-Whitney test.

Table 3. Comparison of laboratory controls of the patient groups with and without TART

Laboratory data	TART		<i>p</i>
	present (<i>n</i> = 9)	absent (<i>n</i> = 29)	
17-OHP (−6/−4), ng/mL	12.5 [0.4 to 43.0] (<i>n</i> = 9)	5.1 [0.1 to 95.0] (<i>n</i> = 26)	0.216 ^a
17-OHP (−4/−2), ng/mL	13.2 [0.5 to 53.5] (<i>n</i> = 9)	5.0 [0.2 to 57.0] (<i>n</i> = 27)	0.338 ^a
17-OHP (−2/0), ng/mL	20.8 [1.6 to 142.8]	9.6 [0.1 to 92.4]	0.188 ^a
Andro (−6/−4), ng/mL	0.5 [0.1 to 6.2] (<i>n</i> = 9)	0.9 [0.3 to 7.0] (<i>n</i> = 26)	0.421 ^b
Andro (−4/−2), ng/mL	1.1 [0.3 to 8.0] (<i>n</i> = 9)	1.0 [0.3 to 8.7] (<i>n</i> = 27)	1.000 ^b
Andro (−2/0), ng/mL	3.4 [0.3 to 6.9]	0.9 [0.3 to 9.1]	0.425 ^b
ACTH (−6/−4), ng/mL	25.0 [10.0 to 124.0] (<i>n</i> = 9)	25.5 [10.0 to 311.5] (<i>n</i> = 26)	0.646 ^a
ACTH (−4/−2), pg/mL	85.8 [10.0 to 194.0] (<i>n</i> = 9)	38.1 [10.0 to 409.0] (<i>n</i> = 27)	0.328 ^a
ACTH (−2/0), pg/mL	67.7 [10.0 to 350.0]	38.2 [10.0 to 344.0]	0.525 ^a
Renin (−6/−4), pg/mL	31.6 [14.4 to 500.0] (<i>n</i> = 9)	38.6 [7.6 to 125.0] (<i>n</i> = 26)	0.735 ^a
Renin (−4/−2), pg/mL	49.5 [11.9 to 201.0] (<i>n</i> = 9)	31.6 [8.4 to 500.0] (<i>n</i> = 27)	0.249 ^a
Renin (−2/0), pg/mL	42.6 [8.4 to 121.3] (<i>n</i> = 8)	42.1 [5.0 to 162.2] (<i>n</i> = 29)	0.909 ^a
LH (−6/−4), UI/L	2.97 [0.3 to 5.8] (<i>n</i> = 5)	2.6 [0.1 to 6.5] (<i>n</i> = 18)	0.881 ^b
LH (−4/−2), UI/L	4.1 [1.6 to 7.3] (<i>n</i> = 4)	2.7 [0.1 to 10.8] (<i>n</i> = 20)	0.333 ^b
LH (−2/0), UI/L	4.5 [3.0 to 8.2] (<i>n</i> = 4)	4.1 [0.1 to 9.2] (<i>n</i> = 22)	0.722 ^b
Inhibin at ultrasonography (pubertal), pg/mL	189.0 [53.0 to 297.0] (<i>n</i> = 5)	218.0 [46.0 to 506.0] (<i>n</i> = 22)	0.190 ^b
17-OHP ↑ (−6/0), %	62.5 [4.8 to 91.2]	43.5 [0 to 85.7]	0.154 ^b
Andro ↑ (−6/0), %	17.4 [0 to 55.2]	17.4 [0 to 71.4]	0.636 ^b
17-OHP ↑ (−2/0), %	80.0 [0 to 100]	40.0 [0 to 100]	0.111 ^b
Andro ↑ (−2/0), %	0.0 [0 to 100]	16.7 [0 to 85.7]	0.686 ^b

Values are given as median [minimum and maximum]. Inhibin, average of inhibin serum levels in pubertal patients; 17-OHP ↑, percentage of 17-OHP increased in relation to the total evaluated dosages; Andro ↑, percentage of Andro increased in relation to the total evaluated dosages. ^a Student's *t* test. ^b Mann-Whitney test.

Table 4. Evaluation of TART presence according to laboratory control treatment groups in the period (–2/0) and crude and adjusted odds ratio

Group	TART presence, <i>n</i>	Total, <i>n</i>	<i>p</i>	OR	95% CI	<i>p</i>	Adjusted OR ¹	95% CI
Clinical form								
SW	8	24	0.096	6.5	0.72–58.90	0.056		
SV	1	14						
17-OHP-GM								
↑	8	24	0.096	6.5	0.72–58.90	0.077		
Normal	1	14						
17-OHP-G10								
↑	5	19	0.703	1.34	0.30–6.02			
Normal	4	19						
Andro								
↑	5	10	0.031	6.00	1.18–30.62	0.018	8.00	1.42–44.92
Normal	4	28						
ACTH								
↑	7	20	0.099	4.31	0.76–24.38	0.170		
Normal	2	18						
Renin								
↑	5	26	0.589	0.64	0.12–3.3	0.131		
Normal	3	11						

17-OHP-GM, group rated in accordance with normal reference value for the method; 17-OHP-G10, group rated in accordance with 17-OHP value <10 ng/mL; ↑, increased; *p*, Wald test probability; OR, crude odds ratio; 95% CI, 95% confidence interval; adjusted OR, odds ratio adjusted by multivariate logistic regression.

¹ We included in the model variables, clinical form, 17-OH-GM, Andro, ACTH, and renin.

Results

There were 42 patients with the classical form of 21-OHD older than 3 years in follow-up. Four patients could not undergo ultrasonography evaluation for personal reasons. Therefore, 38 males were evaluated. Testis ultrasonography revealed paramediastinal bilateral testicular lesions in 9 patients, corresponding to 23.7% (95% CI: 11.4–40.2%).

The mean largest diameter of tumors was 0.89 cm (0.2–2.9) (Table 1). Most of the lesion diameters were smaller than 2 cm, and their diagnosis could only be accomplished by imaging method. The main characteristics of TART patients are presented in Table 1.

No difference was found between patients with and without TART regarding age at treatment onset, follow-up duration, or age at evaluation. There was no difference regarding clinical form, genotypic classification, height, and BMI at ultrasonography (Table 2).

By the time of ultrasound evaluation, 21 patients had attained FH (mean FH SDS – TH SDS = –0.89).

Of the 9 patients that presented with testicular tumors, 4 were prepubertal, 1 was in puberty, and 4 had complet-

ed puberty. Of the 5 patients with testicular lesions who had not reached FH, 4 had bone age advancement according to chronological age (Table 1). TART presence was not different in relation to pubertal stage (*p* = 0.401).

There was also no difference between groups with and without TART regarding the prescribed daily doses of glucocorticoids, serum levels of 17-OHP, Andro, ACTH, renin, and LH (in pubertal patients) in any of the 3 interval periods considered, or in the inhibin B levels at testicular ultrasonography (Table 3).

When assessing the percentages of 17-OHP ≥10 ng/mL (*G* ≥10) and Andro above the upper limit of the method, there was no difference between patients with and without testicular tumors both in the 6-year period (–6/0) of the assessment and in the last 2 years (–2/0) before ultrasonography (Table 3).

Even when the patients were divided into 2 groups of normal or high levels of 17-OHP, considering the reference value of the laboratory method (GM), or the limit value of 10 ng/mL (G10), there was no difference between the individuals with and without TART in any of the 3 periods. The same occurred for ACTH and renin (Table 4).

Nevertheless, in Andro assessments there was a statistical difference between 2 groups in the interval (–2/0) before ultrasonography evaluation. In the group with normal median Andro levels, only 16.7% of patients had tumors, whereas in the group with increased median Andro levels, 50% of patients had lesions (OR = 6.00 [95% CI: 1.18–30.63]). In the other periods, no difference was observed (Table 4).

The predictive variables clinical form, 17-OHP-GM, Andro, ACTH, and renin in the interval (–2/0) were selected for multivariate analysis. After adjustment by logistic regression, only the Andro variable with adjusted OR = 8.00 (95% CI: 1.42–44.92) remained in the model. The chance of TART occurrence in the group with high biannual median Andro levels in the interval (–2/0) was 8 times higher than in the group with normal Andro levels.

There was no correlation between the largest testicular lesion diameter and the percentage of high 17-OHP and Andro, both evaluating the 6-year period (–6/0) ($p = 0.154$ and $p = 0.636$, respectively), or the 2 years (–2/0) previous to the ultrasonography ($p = 0.111$ and $p = 0.686$, respectively).

Discussion

This study showed a prevalence of 23.7% of TART in patients with the classical form of 21-OHD. The presence of tumors in these patients was neither associated with the *CYP21A2* genotype nor with the 21-OHD phenotype, duration of the treatment, presence of puberty, or dose of glucocorticoids prescribed. Other laboratorial parameters such 17-OHP, Andro, ACTH, LH, and renin levels up to 6 years before tumor diagnosis and inhibin B levels at the time of the ultrasonography also did not correlate with TART development. The median Andro level in the 2-year period preceding the diagnosis was the only factor that was significantly associated with TART presence. We found that the probability of developing TART was 8 times higher for the group with increased median Andro values.

We observed that TART development was independent of age, and the youngest patient that presented a tumor was 5.5 years old. Tumors were bilateral in the 9 patients and, in most cases, were small and nonpalpable. These findings are in agreement with the 2009 review by Claahsen-van der Grinten et al. [2] and other recent studies that found bilateral tumors in most patients [29, 30]. Only 1 patient with TART was SV. The 4 prepubertal pa-

tients with TART were SW and carried more severe mutations, classified as N or A, and most of them showed advanced bone age.

The prevalence of 23.7% is similar to that in studies that also included prepubertal patients, such as Avila et al. [31] who described 21% in a study of 19 patients with ages that varied from 6 to 31 years and Claahsen-van der Grinten et al. [32] who found 24% studying 34 patients aged 2–18 years. These authors also identified TART in prepubertal patients.

Compared to other authors [8, 27], however, the prevalence in this study was low. Kang et al. [27] found TART in 64.6% of 48 patients aged 10.6–27.1 years, who had 82.4% of 17-OHP dosages above 10 ng/mL; this percentage was higher than that found in this study in the 6 years before ultrasonography (41.7%). Possibly, the difference occurred because our group had a better control of the treatment.

In a recent study by Kim et al. [29], a lower prevalence of TART (14%) was described in 35 patients; however, they included 3 patients with nonclassic 21-OHD, in which form TART generally does not occur.

The TART prevalence among patients under 10 years of age (25%) in this study was similar to that found by Martinez-Aguayo et al. [6] (21%) and almost half of the lesions in our study were detected in prepubertal patients, which corresponds to a prevalence of 36.3% in this pubertal stage.

Claahsen-van der Grinten et al. [8] did not find lesions in 15 patients younger than 10 years of age. We could not compare our data to the other patients because there was no further information on their clinical form or genotypes. In this study, all patients with TART diagnosis under 10 years of age had mutations that led to more severe enzyme deficiency.

We also believe that the prevalence among pubertal patients in this study (18.5%) is lower than that of Claahsen-van der Grinten et al. [8] probably because these patients had a better control: 10 of 38 patients (26.3%) had high Andro levels in the 2 years prior to ultrasonography, while Claahsen-van der Grinten et al. reported that 19 of the 41 patients (46.3%) had Andro levels above the normal limit.

Considering TART prevalence according to clinical form, no difference was found in this study between SW and SV patients (33.3 and 7.1%; $p = 0.150$), similar to Kang et al. [27] (69.4 and 50.0%; $p = 0.300$).

Differently to Mouritsen et al. [17] and Dumeic et al. [30], but similarly to Claahsen-van der Grinten et al. [8] and Falhammar et al. [33], in this study, 1 patient with

TART had the SV form, while all others had the SW form and carried more severe mutations. Despite the fact that no correlation was observed between clinical forms and TART, we reinforce here the fact that in this study, in the prepubertal stage, we only found TART in patients with more severe mutations, type null and A.

There was also no difference between inhibin B levels in these prepubertal patients with and without TART, just as in the study by Martinez-Aguayo et al. [6]. Although inhibin B levels were lower in pubertal patients with TART, this difference was not significant. Claahsen-van der Grinten et al. [32] also found no inhibin B difference in patients aged 2–18 years with or without TART, probably because gonadal dysfunction would occur later and in those patients with a long time developing tumors.

We studied several laboratory parameters to evaluate the influence of disease control on TART development. The only one that showed a significant difference was the median Andro level of the 2 years preceding ultrasonography. Patients with increased median Andro levels had a higher TART prevalence. These data indicate that hormonal control is a relevant factor in TART development, regardless of pubertal stage. Probably, the influence of puberty, described by some authors, can actually be secondary to the difficulty in controlling the disease during this period, as discussed by Charmandari et al. [34].

Emphasizing the greater influence of the nearest period on TART development, the only patient (patient 2, Table 1) with the SV variant, with group B genotype mutations, who presented testicular tumors at the age of 23 years, had a good disease control until the end of puberty indicated by his FH fully adjusted to the TH. Nevertheless, his disease control was inadequate in the period before ultrasonography, as 62.5% of 17-OHP levels in the 6 years and 100% of them in the 2 years preceding ultrasonography were above 10 ng/mL. Studies have shown that treatment improvement, suppressing ACTH, can lead to tumor regression in the short term and even to normal sperm count [1, 32].

Regarding TART diagnosis in apparently well-controlled patients, it is important to note which hormonal levels may show great variability and that clinical parameters and bone age are of great value in monitoring long-term treatment [35]. It is interesting to point out that 1 prepubertal patient (patient 8, Table 1), diagnosed with TART at 7.5 years of age, had normal 17-OHP, Andro, ACTH, and renin levels in the 2 years prior to ultrasonography, his height SDS being –0.09; however, his bone age was 9.7 years when he was 7.4 years old, which suggests

that good hormonal control occurred only near the laboratory evaluation time.

Although this study shows that laboratory control is implicated in TART genesis, when the cases are individually assessed, we found patients with severe mutations, whose treatments were always inappropriate, and which have no testicular lesions. The explanation for this could be the lack of testicular adrenal rests.

Monitoring patients with the classical form of 21-OHD for early detection and investigating the factors associated with TART development are important because gonadal function in those diagnosed in childhood is apparently maintained at this point; however, it may deteriorate over adult life, with increased tumor size, leading to obstructive infertility [30, 32, 36].

In conclusion, this study shows that TART can be found in prepubertal patients with the classical 21-OHD form and that the poor hormonal control is a factor related to tumor development. Considering that most lesions were smaller than 2 cm, their diagnosis could only be established by imaging methods. Therefore, it is suggested that periodic TART screening by ultrasonography be started at the age of 5 years in all patients with the classical 21-OHD form, especially in poorly controlled cases.

Disclosure Statement

The authors have nothing to disclose.

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