CLINICAL STUDY

Controversies in the evolution of paediatric–adolescent varicocele: clinical, biochemical and histological studies

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Abstract

Objective: To study hormonal and histological parameters of paediatric–adolescent varicocele in order to know certain aspects of its natural history, in an attempt to find prognostic markers of testicular damage.

Design and methods: In a prospective cross-sectional study, we evaluated 93 children and adolescents with left unilateral varicocele and 29 healthy males as control group. All of them were classified according to Tanner stage. Scrotal Doppler in both testes and GnRH and human chorionic gonadotrophin (hCG) tests were performed in all subjects. Surgery was performed in 28 patients and homolateral testicular biopsy in 18.

Results: Hormonal measurements of patients with varicocele were compared with a control group for each Tanner stage. Testicular biopsy specimens were analysed by light and electron microscopy. We only observed statistical differences in Tanner III patients in basal FSH (median and range) controls = 1.70 (1.10–3.70) IU/l vs varicocele = 4.20 (1.00–7.50) IU/l, $P < 0.05$ and in Tanner IV patients in LH post-GnRH: controls = 11.0 (7.50–15.0) IU/l vs varicocele = 18.0 (5.10–29.0) IU/l, $P < 0.05$ and in testosterone post-hCG: controls = 9.50 (7.7–10.0) ng/ml vs varicocele = 12.0 (6.2–23.0) ng/ml, $P < 0.01$. No correlation was found between the various clinical grades of varicocele and hormonal measurements for each Tanner stage. No statistically significant differences were found between pre- and post-operative hormonal findings, either in basal levels or in maximal responses. On the other hand, no morphological abnormalities were observed by electron microscopy in germ cells, tubular wall and interstices.

Conclusions: There appears to be no reliable biochemical marker in children and adolescents that may predict impaired testicular function. A significant size discrepancy between both testes, testicular pain and a hyperresponse to GnRH stimulation should continue to be, for the time being, the indications for surgery.

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Introduction

Varicocele is rare below the age of 10 years and its incidence increases with progressive pubertal development, reaching 15–20% at the age of 14–15 years (1, 2), a rate similar to that reported in adults (3, 4). There are some controversies over the age at which varicocele should be treated. Some authors propose that this pathology be treated in adolescence in order to prevent future fertility problems (5). Their suggestion is based on the fact that one-third of all males evaluated for infertility have a varicocele (6); however, only 15–20% of males with varicocele need treatment for infertility (7). This evidence indirectly shows that most men with varicocele do not have impaired fertility.

The mechanisms by which fertility may be affected are still controversial. Changes in testicular temperature (8), hormonal changes (2–9), the increase in non-collagen proteins and immunoglobulins found in the spermatic vein (7), production of 'toxic substances' (10) and testicular hypoxia (7), have been proposed among other causes. As regards hormonal changes, it is still unknown whether they cause or result from gonadal damage. The information available on circulatory flow alterations (11) and the onset of histological testicular injuries is also limited (12).

The aim of our work has been to study hormonal, circulatory and histological parameters of paediatric–adolescent varicocele to know certain aspects of its natural history, in an attempt to find prognostic markers

of testicular damage in order to select, in the future, the subset of patients requiring surgical treatment.

Subjects and methods

Ninety-three children and adolescents with left unilateral varicocele, chronological ages (CA) ranging between 8.6 and 16.9 years (mean: 12.8 years), and Tanner stages I–V were evaluated. All prepubertal patients were referred by the Healthy Children Section and pubertal patients were referred by the Adolescents Section. In all cases, the Valsalva manoeuvre was part of the routine clinical examination. None of the patients had clinical symptoms consistent with varicocele. Testicular volume was measured by Prader orchidometer. Patients were clinically classified according to the classification of Dubin & Amelar (13) in which grade 1 (G1) corresponds to the small varicocele (detected by Valsalva manoeuvre), grade 2 (G2) to the moderate varicocele (detected by simple palpation) and grade 3 (G3) to the large varicocele (palpable and visible). The control group consisted of 29 healthy males with a CA between 9 and 30 years, and Tanner stages I–V, who were being followed up at our Endocrinology Unit for growth and development control.

In all subjects, a scrotal Doppler study was carried out in both testes, in the standing and supine positions, using ultrasonic monitor vascular Doppler (MV-1, Tecny Med SRL, Buenos Aires, Argentina) with a 5 MHz transducer. The study was performed at the office by one of the authors (M G S) in all outpatients at the first visit. The sonographic findings were characterized as: negative or positive reflux.

A gonadotrophin-releasing hormone (GnRH) test was performed in all subjects (100 µg i.v. bolus) with basal measurements of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone. Both gonadotrophins were also measured 30, 60 and 90 min after GnRH injection. The administration of human chorionic gonadotrophin (hCG) was started 48 h later (at a dose of 1000 IU/day i.m. for 5 days) and a blood sample was taken on the 6th day for testosterone measurement.

LH and FSH were measured by IRMA (Serono Maia Clone, Milan, Italy) and testosterone by RIA. The intra- and interassay coefficients of variation were: LH: 12.1 and 15.2% (mean: 3.4 IU/l) and 5 and 7.5% (mean: 16.5 IU/l); FSH: 9.2 and 15.5% (mean: 4 IU/l) and 5.8 and 5.3% (mean: 13.7 IU/l); and testosterone: 10 and 11.2% (mean: 0.85 ng/ml) and 4.5 and 3.7% (mean: 4.4 ng/ml).

Corrective surgery was indicated in 28 patients, using Palomo's technique (14); left testicular biopsy was performed intraoperatively in 18 of them. The biopsy specimens were processed for both light and electron microscopy. Quantitative analysis was performed by light microscopy determining the presence of tubular lumen,

the maturation state of Sertoli cells and the number of spermatocytes and mature spermatids per cross-tubular section in each biopsy. The volume density (VD) of Leydig cells (i.e. the proportion of the total testicular volume occupied by Leydig cells) was evaluated by the differential point-counting method (15) employing a Zeiss point-counting eyepiece (Carl Zeiss, Oberkochen, Württ, Germany). Since an estimate of testicular volume was obtained, the absolute Leydig cells amount (mg) per testis could be calculated. The results of the stereological and cell-counting methods are shown in Table 2. Electron microscopy was used to analyse Sertoli cell morphological maturation signs (tripartite nucleolus, folded nuclear membrane, intercellular junctions), Sertoli cell abnormalities (vacuolation), tubular wall maturation (abnormal cells, basement membrane thickening), spermatogonia (infantile or adult characteristics) and Leydig cells (identifiable precursors, adult cells, involutional cells). The GnRH and hCG tests were repeated in 14 patients 6 months after surgery. Informed consent was obtained from all subjects and their parents for hormonal studies and for biopsies when there was an indication for surgery. The study protocol was submitted to the Alvarez Hospital Education and Research Committee and to the Ethical Committee.

The Mann–Whitney test was used to compare hormonal findings in patients with varicocele and controls for each Tanner stage. The Wilcoxon signed rank test was used for evaluation of pre-surgery vs post-surgery findings (16).

Results

Clinical findings

Out of 93 patients, 17 were at Tanner stage I (G1 varicocele: ten; G2: two and G3: five), 18 at Tanner stage II (G1: five; G2: five and G3: eight), 16 at Tanner stage III (G1: four; G2: four and G3: eight), 29 at Tanner stage IV (G1: nine; G2: twelve and G3: eight) and 13 at Tanner stage V (G1: none; G2: six and G3: seven).

Out of 29 controls, nine were at Tanner stage I, five at stage II, five at stage III, five at stage IV and five at stage V.

As regards the Doppler study, all patients with varicocele had positive reflux, while no reflux was observed in any of the subjects of the control group.

Out of 28 patients who underwent surgery (all of them G3 varicocele) three were at Tanner stage I, four at stage II, five at stage III, five at stage IV and five at stage V.

Out of 93 patients, 44 who had not undergone surgery were clinically followed until they reached an adult testicular volume, with no worsening of varicocele. The remaining 21 patients who had not been operated on were dropouts. As regards the testicular volume of these 65 patients at the start, in prepubertal patients the difference between the affected testis and

Table 1 Basal levels and maximal response of LH (LH_B and LH_{Mx}) (IU/l) and FSH (FSH_B and FSH_{Mx}) (IU/l) to GnRH, and basal levels and response of testosterone (T_B and T_{Post}) (ng/ml) to hCG in control subjects and varicocele patients at the different Tanner stages. Values are expressed as median and range.

Tanner stage	Subjects	LH _B	LH _{Mx}	FSH _B	FSH _{Mx}	T _B	T _{Post}
I	Controls (<i>n</i> = 9)	0.40 (0.40–0.90)	2.15 (0.63–12.0)	1.45 (0.40–3.00)	4.20 (2.40–5.90)	0.10 (0.10–0.30)	3.20 (1.20–4.90)
	Varicocele (<i>n</i> = 16)	0.40 (0.40–2.10)	3.80 (1.10–13.0)	2.08 (0.40–4.50)	4.80 (2.60–10.0)	0.20 (0.10–1.50)	2.50 (0.80–11.50)
II	Controls (<i>n</i> = 5)	1.25 (0.40–1.90)	11.0 (2.80–18.0)	1.80 (0.40–3.10)	10.0 (0.40–15.0)	1.50 (0.10–2.30)	7.60 (3.00–10.60)
	Varicocele (<i>n</i> = 14)	1.60 (0.40–3.30)	13.0 (8.00–20.0)	2.90 (0.85–5.80)	5.60 (2.35–14.0)	1.20 (0.35–4.70)	8.40 (2.50–13.10)
III	Controls (<i>n</i> = 5)	2.60 (1.50–3.00)	13.5 (7.60–16.5)	1.70 (1.10–3.70)	4.20 (1.60–5.80)	3.60 (2.20–5.60)	9.50 (5.40–13.00)
	Varicocele (<i>n</i> = 14)	1.50 (0.40–2.70)	15.0 (5.60–28.0)	4.20* (1.00–7.50)	6.20 (1.00–12.6)	3.40 (0.25–5.80)	9.80 (5.20–18.00)
IV	Controls (<i>n</i> = 5)	1.40 (0.75–3.10)	11.0 (7.50–15.0)	3.60 (2.20–7.10)	7.50 (4.90–11.0)	3.35 (3.20–6.80)	9.50 (7.70–10.00)
	Varicocele (<i>n</i> = 29)	2.40 (0.75–7.20)	18.0* (5.10–29.0)	3.60 (1.00–8.00)	6.00 (2.50–37.0)	4.80 (2.50–7.30)	12.00** (6.20–23.00)
V	Controls (<i>n</i> = 5)	3.50 (1.90–4.50)	18.0 (11.0–23.6)	3.70 (1.80–4.10)	5.00 (3.80–6.60)	4.80 (4.40–8.90)	12.00 (8.00–17.00)
	Varicocele (<i>n</i> = 9)	2.15 (0.60–3.90)	14.0 (10.0–17.0)	3.30 (1.50–11.0)	6.00 (2.40–16.0)	4.50 (3.50–6.20)	11.45 (6.60–14.00)

P* < 0.05, *P* < 0.01. Mann–Whitney test.

the contralateral testis did not exceed 50%; in pubertal patients the difference between the two testes was less than 2 ml. Out of 28 operated patients, 13 showed a favourable evolution, reaching an adult testicular volume. Fifteen of the operated subjects dropped out between 2 and 10 months after surgery, with no testicular evolution.

Biochemical findings

Basal levels of LH, FSH and testosterone and their

maximal response to GnRH and hCG respectively are shown in Table 1.

Basal FSH levels at Tanner stage III and the post-stimulation maximal response of LH and testosterone in stage IV were significantly higher than those of the control group. No statistically significant differences in basal LH, basal testosterone and the maximal response of FSH were found between subjects with varicocele and the control group in any of the Tanner stages.

No relationship was found between the various

Table 2 Paediatric-adolescent varicocele: histological findings by light microscopy.

Tanner stage	Testicular volume (ml)	VD Leydig cells (%)	Leydig cells/testis (mg)	Mature spermatides/tubule	Spermatocytes/tubule	Lumen	Sertoli	Complete spermatogenesis
I	3	0.07	2	0.0	0.0	–	Immature	–
I	2	0.28	5	0.0	0.0	–	Immature	–
II	4	0.60	24	7.1	0.0	–	Immature	–
II	5	1.62	81	2.3	0.0	+	Intermediate	–
III	6	2.83	170	3.8	0.0	+	Intermediate	–
III	7–8	2.13	106	+	6.8	+	Mature	+
III	7	1.56	109	+++	0.7	+	Mature	+
III	6	1.83	110	+++	10.7	+	Mature	+
III	8	1.69	135	+	9.0	+	Intermediate	+
III	6	2.63	158	+	5.1	+	Intermediate	+
V	15	1.92	288	+++	20.6	+	Mature	+
IV	9	3.23	290	+++	12.3	+	Mature	+
V	12	2.79	335	+++	17.6	+	Mature	+
V	11	3.07	338	+++	14.7	+	Mature	+
IV	10	3.76	376	+++	18.7	+	Mature	+
V	12	3.18	381	+++	12.7	+	Mature	+
IV	10	4.45	445	+++	12.8	+	Mature	+
V	20	2.58	516	+++	23.0	+	Mature	+

clinical grades of varicocele or Doppler patterns and hormonal findings for each Tanner stage ($P = \text{NS}$).

Surgery

All operated patients ($n = 28$) had G3 varicocele. In pubertal patients, the difference in testicular volume between the two testes was greater than 2 ml. In prepubertal patients, a difference in size between the affected testis and the contralateral testis was considered when it was greater than 50%.

In 14 out of 28 patients who were operated on, hormonal tests were repeated 6 months after surgery. Biochemical findings are shown in Fig. 1. No statistically significant differences were found between pre- and post-operative findings, either in basal levels or in maximal responses.

Pathological anatomy

Histological findings did not differ from those described in testicular development during normal puberty (Table 2). No morphological abnormalities were observed by electron microscopy in germ cells, tubular wall and interstice (Fig. 2).

Discussion

The management of varicocele in adolescents is still controversial. Because of the impaired fertility of affected males, they are considered as an at-risk group (17). Varicocele may be associated with alterations in testicular trophism, abnormal testicular histology and gonadal function impairment (18–21). However, there are not enough data about the time at which this deterioration begins.

One of the controversial issues is establishing the potential existence of prognostic markers that would make it possible to decide for surgery and to determine, when necessary, the appropriate time to perform it (22, 23). In our opinion, a better knowledge of the natural history of the disease might help to resolve this dilemma. For this reason, in our study we have evaluated different clinical, biochemical, circulatory and histological parameters in prepubertal and pubertal patients, and, by correlating them, we have made an attempt to analyse their potential usefulness. However, it is worth mentioning that since our patient population could not be followed up until adulthood, it was not possible to find clear prognostic markers of testicular damage.

The clinical findings of the present work show that 59% of prepubertal patients had small varicoceles, which were not observed in any of the patients at Tanner stage V, while large varicoceles were present in 54% of patients at Tanner stage V and only in 29% of prepubertal subjects. From this data we could infer two concepts: (i) the high percentage of small varicoceles in

Figure 1 Pre- and post-surgical findings in paediatric-adolescent varicocele. ● Pre-surgery, ▲ post-surgery. B = basal, Mx = maximal response post GnRH, Post = post-hCG. Pre-surgery vs post-surgery $P = \text{NS}$ (Wilcoxon signed rank test).

Figure 2 Specimen obtained from biopsy performed in a patient at Tanner IV, testicular volume 10 ml (electron microscopy). (a) Seminiferous tubule section with two mature spermatogonia and one spermatocyte (6700 $\times$). (b) Interstices section with two mature Leydig cells (8000 $\times$).

prepuberty could explain, at least partially, the reason why this pathology may be underdiagnosed in this period, and (ii) the worsening of varicoceles between prepuberty and the final stage of sexual maturation. Because of the high dropout rate, no definitive conclusions could be drawn on the follow-up. Probably the high dropout rate may be attributed to the lack of comprehension of the importance in the parents and/or to non-cooperation of the general practitioners.

Some years ago, in a study on pubertal varicocele (11) we found a significant increase in LH response to GnRH and an increase in testosterone post-hCG stimulation, in some unilateral varicoceles. Nevertheless, we pointed out that it would be advisable to divide the possible findings according to the stage of sexual maturation. Later, while studying prepubertal patients (24) we noticed that basal levels of LH and FSH in large varicoceles were significantly higher than those of the control group, with no hyperresponse to GnRH, and no alterations in basal and post-hCG levels of testosterone. Our hypothesis was that those increases might represent autocrine and paracrine alterations in the regulation of testicular function in those patients. In the present work, having increased the number of patients enrolled in the study, considering subjects with unilateral varicoceles ranging from prepuberty to Tanner stage V, we noticed some changes in basal levels of FSH, and in the maximal response of LH and testosterone post-stimulation in the midstages and final stages of puberty. These changes did not follow a consistent pattern when we made an attempt to correlate them with the clinical grades of varicocele. This would show that a certain clinical grade of varicocele does not necessarily imply a specific biochemical alteration, so that a hormonal evaluation is required for each individual case.

The evaluation performed in our patients 6 months after surgery did not show significant hormonal changes. This might be partly explained by the pubertal evolution of these patients, whose physiological hormonal changes may have masked possible hormonal changes between pre- and post-operative evaluations.

An important contribution of our work has been the study of biopsy specimens from our operated patients by light and electron microscopy. Surprisingly, we did not find alterations in the study with light microscopy, which was confirmed by the normal findings obtained in the study with electron microscopy. This could be possibly attributed to the fact that biopsies were performed at an early stage of varicocele evolution, and before the occurrence of injuries, which are partly time-dependent. Histological lesions may possibly be evidenced during periods of more active spermatogenesis. Moreover, some authors only found histological abnormalities in adolescents having a hyperresponse post-GnRH stimulation (12). This suggests that the hormonal changes occurring in some cases might precede histological injuries and this could partly

explain the absence of histological damage in our patients.

In conclusion, varicocele is an heterogeneous entity with marked individual variations. At present there is no reliable biochemical marker that may predict impaired testicular function. Therefore, a significant size difference between both testes, testicular pain and a hyperresponse to GnRH stimulation should continue to be, for the time being, the indications for surgery. Hormonal changes and histological injuries will be, to a large extent, time-dependent, in many cases worsening during puberty. Perhaps, in the future, the design of new research methodologies and the use of ultrasensitive biochemical assays will contribute to the prospective characterization of the subset of patients at risk of testicular damage.

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