Journal of Pediatric Surgery xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg

Structural study of the cremaster muscle in patients with retractile testis

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ARTICLE INFO

Article history:
Received 13 February 2017
Received in revised form 16 March 2017
Accepted 23 April 2017
Available online xxxx

Key words:
Cremaster muscle
Retractile testis
Cryptorchidism
Histology

ABSTRACT

Objective: To analyze the structure of the cremaster in patients with retractile testis (RT), comparing the distribution of nerves, elastic system and muscles with patients having cryptorchidism and inguinal hernia (IH).
Patients and methods: We studied 31 patients, 17 with RT (mean age = 5.17years); 9 with IH (mean age = 2.6) and 5 with cryptorchidism (mean age = 3). A cremaster biopsy was performed and submitted to routine histological processing and studied using histochemistry and immunohistochemistry. The samples were photographed under an Olympus BX51 microscope. The images were processed with the Image J software and the cremaster muscle structures were quantified. Means were compared statistically using ANOVA and the unpaired t-test ($p < 0.05$).
Results: There were no differences ($p = 0.08$) in diameter of muscle fiber between the groups. The muscle fiber density differed between patients with RT and IH ($p = 0.02$): RT (mean = 17.71%, SD = 16.67), IH (mean = 38.06%, SD = 14) and cryptorchidism (mean = 21.47%, SD = 16.18). There was no difference ($p = 0.07$) in the density of elastic fibers in the three groups. We observed a lower concentration of cremaster nerves of patients with RT compared with IH ($p = 0.0362$): RT (mean = 1.72%, SD = 0.58), IH (mean = 3.28% SD = 0.94) and cryptorchidism (mean = 2.52%, SD = 0.53).
Conclusions: Retractile testis is not a normal variant, and presented a similar cremaster muscle structure as in patients with cryptorchidism.
Level of evidence: II; prospective comparative study.

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Retractile testis is defined as a suprascrotal testis that can be manipulated into the scrotum and will remain there without traction until the cremasteric reflex acts [1]. The management of this condition is still controversial.

Recent articles have recommended only observation of retractile testis cases [2,3]. In more than 70% of patients with retractile testis, the condition evolves favorably without the need for surgery [1]. However, a 25% risk exists of these testes ascending and becoming cryptorchidic in adolescence [4].

Structural and ultrasound studies have demonstrated morphological alterations in the germinal epithelium and Sertoli cells of retractile testes [5,6]. A study of young adults who had retractile testis during prepuberty showed that only 28.8% of the patients had normal spermogram [7].

Other studies have analyzed and compared the structural alterations of the cremaster muscle in patients with cryptorchidism, hydrocele and inguinal hernia [8–10]. However, studies of the structure of the cremaster muscle in patients with retractile testes are rare in the literature.

We hypothesize that the patients with retractile testis present structural alterations in the extracellular matrix, muscles and nerves in the cremasteric muscle. We propose to test these hypotheses by evaluating the structure of the cremaster muscle in patients with retractile testis and quantitatively compared the distribution of the nerves, elastic system and musculature of patients suffering from cryptorchidism and inguinal hernia.

1. Methods

The experimental protocol described herein was approved by the Ethical Committee for Human Experimentation of our University. This prospective study was carried out in accordance with the ethical standards of the hospital's institutional committee on human experimentation.

From January 2016 to January 2017, we studied 31 patients with testicular pathologies who had undergone surgery at our hospital. Of them 17 presented true retractile testis, with age between 1 and 10 years (mean = 5.17); 9 had inguinal hernia, with age between 1 and 7 years (mean = 2.6) and 5 had cryptorchidism, with age from 1 to 4 years (mean = 3). All the patients with retractile testis were surgically treated through an incision in the raphe of the scrotum, while in the other patients the operation involved a transverse incision in the inguinal region. All 31 patients had unilateral testicular anomalies.

The retractile testis in this sample was defined based on physical examination findings. In 17 cases included in this study the retractile testis can be brought down into the scrotum without tension and, after gentle

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http://dx.doi.org/10.1016/j.jpedsurg.2017.04.013
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Please cite this article as: Favorito LA, et al, Structural study of the cremaster muscle in patients with retractile testis, J Pediatr Surg (2017), http://dx.doi.org/10.1016/j.jpedsurg.2017.04.013

massaging of the cord, stay there upon release for a while. The boys with retractile testis need periodic follow-up. A great number of our patients have a difficult economic situation and live far from our institution. So, the surgical indication is preferred instead of monitoring [11].

During the surgical procedure, at the moment of dissecting the testis and spermatic cord, tissue samples were obtained from the cremaster muscle for biopsy. Smooth muscle, connective tissue and elastic fibers were studied by histochemical and immunohistochemical methods.

The samples were processed by routine laboratory techniques, involving dehydration in successive alcohol baths, diaphonization in xylene and embedding in paraffin. From these blocks, sections were cut with thickness of 5 μm at intervals of 200 μm and subjected to different staining. For the histochemical analysis, the sections were stained with hematoxylin and eosin to confirm the material's integrity, Masson's trichrome for observation and quantification of the muscle tissue, and Weigert's resorcin fuchsin after oxidation by oxone to observe the elastic system fibers. The immunohistochemical analysis of the cremaster nerves was performed with tubulin (tubulin, beta III, mouse monoclonal antibody). The histochemical and immunohistochemical analysis was performed by a single pathologist.

1.1. Morphometry

All the images were captured by an Olympus SD70 camera coupled to an Olympus BX51 microscope (Fig. 1A). The images were processed with the ImageJ software, using its plugin (http://rsb.info.nih.gov/ij/), by which the cremaster muscle structures were quantified by the stereological method [12–15]. We studied 5 microscopic fields chosen at random, totaling 25 test areas studied for each cremaster sample for the quantitative analysis. We used the Image J software, version 1.46r, loaded with its own plug-in (http://rsb.info.nih.gov/ij/). All sections were photographed with a digital camera (DP70, Olympus America, Inc., Melville, New York) under the same conditions at a resolution of 2040 1536 pixels, directly coupled to the microscope (BX51, Olympus America, Inc.) and stored in a TIFF file. To quantify the smooth muscle tissue we used the Color Segmentation of Image J software, where the program selected structures of different colors and calculates the amount of each component. The muscle fiber diameters were also determined using the "straight" tool of the ImageJ program, which involves marking a straight line between the two sides of a fiber and measuring the distance (Fig. 1B).

For quantifying the elastic fibers and nerves we used the Image J software to determine the volumetric density (Vv) of each component. Results for each field were obtained through the quantification assessment method, by superimposing a 100-point test grid (multipurpose test system) on the video monitor screen. The arithmetic mean of the quantification in 5 fields of each section was determined. Afterwards, we obtained the mean quantification value for the 5 sections studied from each cremaster sample (total of 25 test areas).

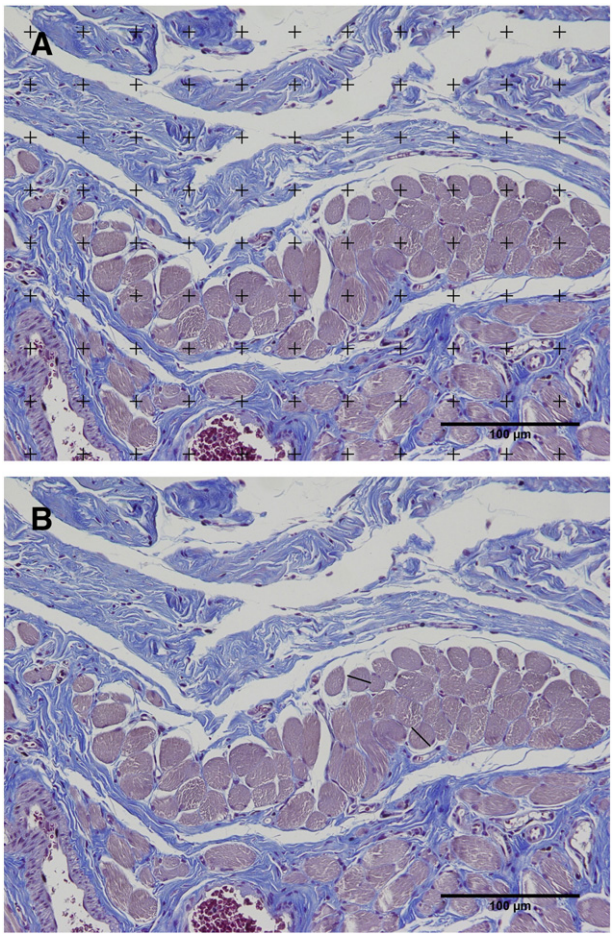

Fig. 1. Morphometric analysis of the cremaster muscle. A) Quantification of the muscle tissue with the ImageJ software using a 100-point grid. 10-year-old patient with retractile testis. Masson's trichrome ×200. B) Measurement of the muscle fiber diameter using the "straight" tool of the ImageJ software, where a straight line (black) is marked between the two ends a muscle fiber and the distance is measured. 10-year-old patient with retractile testis. Masson's trichrome ×200.

1.2. Statistical analysis

We applied one-way ANOVA with the Bonferroni post-test for statistical analysis, using the Graph Pad Prism software, with significance of $p < 0.05$. Means were compared statistically using ANOVA and the unpaired t-test, also with $p < 0.05$.

2. Results

The results of the stereological quantification of the muscle fiber diameter, the muscle fiber density, the density of elastic fibers and the cremaster muscle nerves of the three groups studied are reported in Table 1.

The quantitative analysis did not show a significant difference ($p = 0.08$) in the muscle fiber diameter, measured in micrometers (μ), among the three groups: retractile testis (mean = 22.80 μ; SD = 9.84), inguinal hernia (mean = 22.76 μ; SD = 3.01) and cryptorchidism (mean = 24.75 μ; SD = 7.02). However, the muscle fiber density was significantly different between the patients with retractile testis and those with inguinal hernia ($p = 0.02$): retractile testis (mean = 17.71%; SD = 16.67), inguinal hernia (mean = 38.06%; SD = 14) and cryptorchidism (mean = 21.47%; SD = 16.18).

The quantitative analysis also did not show significant difference ($p = 0.07$) in the density of elastic fibers among the three groups: retractile testis (mean = 8.9%; SD = 2), inguinal hernia (mean = 8.92%; SD = 1.55) and cryptorchidism (mean = 10.47%; SD = 1.95).

Finally, the quantitative analysis of the cremaster muscle nerves showed a significantly lower concentration of nerve fibers in patients with retractile testis compared to those having inguinal hernia ($p = 0.0362$): retractile testis (mean = 1.72%; SD = 0.58), inguinal hernia (mean = 3.28%; SD = 0.94) and cryptorchidism (mean = 2.52%;

Table 1
The table shows the mean (M) and standard deviation (SD) of the stereological quantification, of muscle fiber diameter (Fiber), in micrometers; muscular density (Musc), elastic system (Elastic) and quantification of nerves (Nerve) in the three groups studied.

Group	Mean age (years)	Fiber M (μ)/SD	Musc M(%)/SD	Elastic M(%)/SD	Nerve M(%)/SD
Retractile testis	5.1	22.8/9.8	17.7/16.6	8.9/2	1.7/0.5
Inguinal hernia	2.6	22.76/3.0	38/14	8.9/1.5	3.2/0.9
Cryptorchidism	3	24.75/7.0	21.4/16.1	10.4/1.9	2.5/0.5
Statistical analysis	–	$p = 0.08$	$p = 0.02$	$p = 0.07$	$p = 0.0362$

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