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The efficacy of topical testosterone treatment on preputial microvessel density in distal hypospadias

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Aim: To investigate the efficacy of topical testosterone treatment on the microvessel density (MVD) of hypospadiac prepuce.

Materials and methods: The prepuces of 20 healthy children undergoing routine circumcision served as the control group, while the prepuces of 20 children undergoing hypospadias repair served as the hypospadias group. The hypospadias group was further divided into 2 equal subgroups (n = 10) as the hypospadias with or without testosterone treatment groups. The meatal locations were coronal and subcoronal in all of the patients with hypospadias. The prepuces were stained by immunohistochemical methods using panendothelial cell antigen CD31 in order to assay their MVD. The statistical analysis among the groups was done using one-way analysis of variance.

Results: The MVD was significantly lower in the hypospadias without treatment group than in the control group (37.16 ± 18.20 vs. 58.75 ± 24.98; P = 0.037). Preoperative testosterone treatment increased the mean MVD to 51.92 ± 10.21 in the treatment group, but it was not statistically significant (P = 0.116).

Conclusion: Topical testosterone treatment prior to hypospadias surgery restores defective MVD in the prepuce, but this desired effect is unsatisfactory with a single application of a high dose of testosterone.

Key words: Hypospadias, testosterone, prepuce, microvessel density

Introduction

Preputial vascularity is crucial for hypospadias repair, because the prepuce is used for the reconstruction of the neourethra and penile skin coverage (1). The vascular anatomy of the prepuce is also important when choosing the operative technique in hypospadias, and a better knowledge of preputial vascular anatomy may improve the surgical outcome (2). The morphological characteristics and the vascular anatomy of the hypospadiac prepuce, and the correlation among them, have been previously documented (1,3). The defective vessel pattern of the hypospadiac prepuce has also been shown (2). Microvessel density (MVD) has been used for vascular analysis in tissue samples for several decades. In this respect, we compared the vascularity of normal and hypospadiac prepuces using MVD in our previous study, and we demonstrated the defect in the MVD of the hypospadiac prepuce and its negative correlation with the severity of hypospadias (4). Topical or intramuscular preoperative testosterone treatment has been used to increase the penile length in hypospadiac children with micropenis in order to permit easier surgical repair and to decrease the postoperative complications (5,6). Increased preputial vascularity is important for the final outcome in hypospadias surgery. Testosterone stimulates angiogenesis and vascular regrowth (7).

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Thus, a prospective clinical trial was designed in order to clarify the impact of preoperative topical testosterone treatment on preputial MVD.

Materials and methods

Permission from the institutional review board was obtained before this clinical trial (IRB approval: 2177-TU-10). Informed consent was obtained from one of the parents of each patient before the study.

Study groups

The prepuces of 20 children, aged between 2 and 12 years (mean: 5.65 ± 3.03 years) with distal hypospadias undergoing surgical repair, were included in the study. The prepuces of 20 healthy age-matched (mean: 5.10 ± 3.30 years) children undergoing routine circumcision served as the control group. The hypospadias group was further divided into 2 equal subgroups (n = 10) as the hypospadias with or without treatment groups. No randomization was performed between the hypospadias subgroups. The patients who required penile lengthening were included in the treatment group, and an ampoule of testosterone (Sustanon, 1 mL, 250 mg; Schering Plough) was applied topically to the penis and prepuce of the patients 1 month before the operation. The meatal locations were coronal and subcoronal in all of the patients with hypospadias. Cases with glanular hypospadias and redo operations were excluded from the study. None of the control subjects had suffered an attack of balanitis before circumcision. The same surgeon operated on all of the patients. The most distal part of the inner preputial layer from the dorsal side of the penis was harvested for histological evaluation.

Immunohistochemistry

Immunohistochemical staining was carried out with the avidin–biotin–peroxidase system using a monoclonal antibody (CD31/PECAM-1, clone JC/70A; Lab Vision Corp., Fremont, CA, USA) against the panendothelial cell antigen CD31 (platelet/endothelial cell adhesion molecule). Briefly, 4-μm-thick consecutive sections were deparaffinized and hydrated through a graded series of alcohol. After the inhibition of endogenous peroxidase activity by immersion in 3% H_2O_2/methanol solution, the antigen retrieval was conducted using 10 mmol/L of citrate buffer (pH 6.0) in a microwave oven for 10 min at 120 °C. The sections were incubated with primary antibodies, thoroughly washed in phosphate-buffered saline, and then incubated with biotinylated secondary antibody followed by the avidin–horseradish peroxidase complex (Lab Vision). Finally, the immune complexes were visualized by incubation with DAB chromogen (Lab Vision), and nuclear counterstaining was accomplished with Mayer's hematoxylin. As a negative control, appropriately diluted nonimmune sera were applied instead of the primary antibody. All of the negative controls showed low background staining (data not shown).

Determination of microvessel density

The preputial microvessels included capillaries and small venules and arterioles. When measuring the MVD, single endothelial cells were excluded because they cannot be considered as microvessels. The presence of a vascular lumen was not necessary to identify a microvessel. Care was taken to select microvessels, i.e. capillaries and small venules, from all of the CD31-stained vessels. They were identified as transversally sectioned tubes with a single layer of endothelial cells, either with or without a thin basement membrane. Using a light microscope (Olympus CX 41, Hamburg, Germany), areas of high-density staining were identified in low-power (100×) fields by 2 pathologists who were blinded to the patient groups. Most of the CD31-positive microvessels were identified in this area, which thus resembled an "extended hot spot" of angiogenesis. Neovascularity was counted in 5 random high-power (200×) fields within these hot spots. The mean results were recorded for analysis.

Statistical analysis

All of the data are reported as means ± 1 standard deviation. Data were analyzed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test confirmed that the data were distributed normally ($P = 0.211$; >0.05 confirms normal distribution), and Levene's statistic test of homogeneity confirmed that the data were nonhomogeneous ($P = 0.001$; >0.05 confirms homogeneity of variances). Thus, statistical analysis among the groups was done by one-way analysis of variance followed by Dunnett's T3 test as a post hoc for the pairwise comparisons. All of the tests were 2-sided and $P < 0.05$ was considered statistically significant.

Results

The MVDs of the groups are shown in Figure 1. The MVD was significantly lower in the hypospadias without treatment group than in the control group (37.16 ± 18.20 vs. 58.75 ± 24.98; P = 0.037). Although it was not statistically significant (P = 0.116), preoperative treatment with topical testosterone increased the preputial MVD in the treatment group when compared to the hypospadias without treatment group (51.92 ± 10.21 vs. 37.16 ± 18.20) (Figure 2).

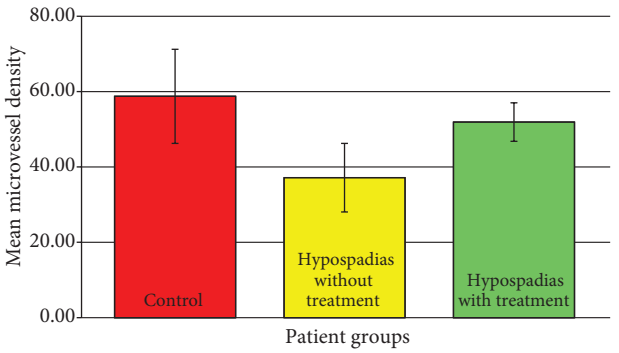

Figure 1. The MVDs of the groups. The MVD was significantly lower in the hypospadias without treatment group than in the control group (P = 0.037). Although it was not statistically significant (P = 0.116), preoperative treatment with topical testosterone increased preputial MVD in the treatment group.

Discussion

The prepuce is used for reconstruction of the neourethra and penile body skin in hypospadias surgery. Therefore, a well-vascularized prepuce is extremely important for the outcome of the surgery. Perovic and Radojicic (1) investigated the development, course, and distribution of the preputial blood vessels in hypospadiac patients, and they applied the results of the study to the surgical correction of the pathology. They showed that the prepuces were well vascularized, with a predominant 1 or 2 blood vessels in 70% of the patients. However, a net-like vascularization with no predominant blood vessels was found in 30% of the patients. They concluded that a prepuce with a net-like vascularization without 1 or 2 predominant arteries was unsuitable for preparing well-vascularized preputial flaps for reconstruction of the neourethra. However, the surgical outcome of the patients (complication rates) and its correlation with the vascular pattern of the prepuce were surprisingly absent in the results of their study.

In another study, morphological classification, along with the vascular pattern of hypospadiac prepuces and the impact of the surgical outcome, was done. This showed a significant correlation between the morphology and vascularization of the prepuces. Underdeveloped prepuces and those with an unfavorable vascular pattern used for urethroplasty had a higher percentage of complications (3). Yucel et al. (2) compared the blood vessel patterns of hypospadiac and normal patients. They showed that a net-like arterial system was more common in hypospadiac patients (47%). They concluded that the identification of the vascular anatomy of the hypospadiac prepuce prior to reconstruction of the neourethra with preputial flaps may improve the surgical outcome, especially in proximal cases. Soyer

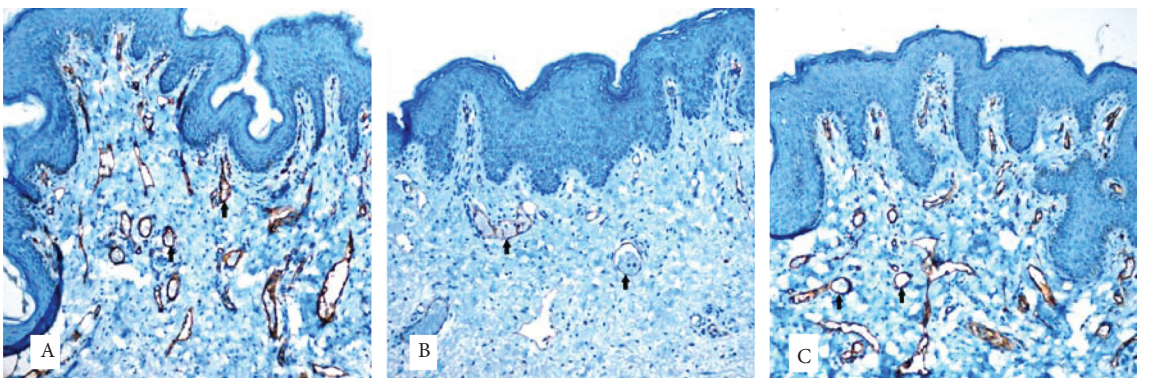

Figure 2. The microscopic appearance of the microvessels (200×): A) Normal immunohistochemical CD31 staining of microvessels in the control group, as marked by arrows; B) arrow indicates the decreased preputial microvessels in a hypospadiac patient without testosterone treatment; and C) testosterone treatment increased the preputial microvessels.

et al. (8) revealed that not only the vascular anatomy but also angiogenesis may be defective in hypospadiac prepuces. The results of these studies were very suggestive in order to investigate the defective preputial MVD in children with hypospadias. Thus, the MVD of the hypospadiac prepuce was investigated to evaluate neovascularization by means of immunohistochemical methods, using a monoclonal antibody against the panendothelial cell antigen CD31, in our previous study. Our results demonstrated that the MVD of the hypospadiac prepuces was significantly lower than that of the healthy controls, and a gradual decrease in the MVD was also observed in proportion to the severity of the hypospadias (4).

Testosterone treatment prior to hypospadias surgery in order to increase penile length has been shown to be beneficial (5,6). In an experimental transplant model, the treatment of human foreskin with testosterone increased vascularity and decreased early fibrosis (9). In another experimental study, postoperative administration of testosterone in rabbits following hypospadias repair changed the histologic composition of the urethral epithelium and led to an exaggerated inflammatory response in the supportive stroma (10). More recently, the application of 1% testosterone propionate ointment before hypospadias surgery was shown to induce neovascularization, increasing the absolute number of vessels and blood volume density (11). In this study, topical testosterone was applied twice daily for 30 days. Nevertheless, we applied a single high topical dose of testosterone 1 month before the operation. We also used an injectable form of testosterone for topical treatment. The absorption of this form may be insufficient for hormonal stimulation. This short and insufficient stimulation is probably responsible for the statistically insignificant difference in MVDs between the hypospadias subgroups in our study.

Preoperative topical testosterone treatment increased the neovascularization of the prepuce in patients with hypospadias, but this desired effect is unsatisfactory with a single application of testosterone. The number of patients and the degree of hypospadias (only distal cases) were the limitations of the present study. Larger series with more severe cases and repeated doses are needed in order to clarify the effects of preoperative topical testosterone treatment on preputial MVD.

Acknowledgments

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