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The correlation between preputial blood flow and microvessel density in distal hypospadias: A prospective clinical study

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Abstract *Objective:* A prospective clinical study was designed to investigate the correlation between preputial blood flow (BF) and microvessel density (MVD).
Patients and methods: A total of 44 children were included in the study. The hypospadias group consisted of 16 children undergoing distal hypospadias repair, and the control group consisted of 28 age-matched healthy children undergoing circumcision. BFs were measured using a laser Doppler flowmeter on the most distal part of the dorsal prepuces, and then the tissue samples were harvested from the same location. They were immunostained with an antibody against CD31 in order to assay MVD. The statistical analyses were carried out using Student's *t* test and Pearson's correlation analysis.
Results: The preputial MVD was found to be significantly decreased in the patients with hypospadias compared with the healthy children (33.95 ± 9.79 vs. 48.25 ± 10.08; $p < 0.05$), whereas there was no difference in terms of the BF (40.58 ± 16.16 vs. 33.09 ± 19.65; $p > 0.05$).
Conclusions: We found no correlation between the preputial MVD and BF in the present study. This result suggests that reduced preputial MVD does not have any influence on BF in distal hypospadias.

Introduction

Hypospadias is one of the most common congenital urogenital anomalies. Numerous surgical techniques have been described to date. Although the urethral plate is used in most cases, the prepuce is used successfully to create neourethra in some cases where the urethral plate itself is not wide enough. The decreased microvessel density (MVD)

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(which means a defective vessel pattern) of the hypospadiac prepuce has been shown recently [1]. The effect of this defective vascular pattern on preputial blood flow (BF) still remains unclear. In this study, we hypothesized that there might be a positive correlation between defective preputial vascular pattern and BF in patients with hypospadias, and designed a prospective clinical study to clarify this hypothesis. For this purpose, we used *laser Doppler flowmetry (LDF), which* is an accurate and reliable method for assessing microcirculatory function in experimental and clinical researches [2], to measure preputial BF.

Patients and methods

Permission from the institutional review board was obtained before this clinical trial (IRB number: 2010—4-2). Informed consents were obtained before the study.

Study groups

A total of 44 children were included in the study between April 2010 and September 2012. The hypospadias group consisted of 16 children undergoing distal hypospadias repair, and the control group consisted of 28 age-matched healthy children undergoing circumcision. Cases with redo operations and previous history of balanitis xerotica obliterans, balanitis, posthitis, or hormone therapy were excluded from the study. All measurements and surgical procedures were performed in the operating room under general anesthesia by the same surgeon.

Measurement of preputial blood flow

The preputial BF was measured using a laser Doppler flowmeter (PF 5001, Perimed company, Järfälla, Sweden) before making any surgical intervention to the preputium. The laser Doppler probe (Probe 407 small straight probe, Perimed) was initially fixed on the most distal outer part of the dorsal preputium without prominent vascularity by a double-sided tape in both groups, and then 10 consecutive measurements were noted. All measurements were expressed as perfusion units (PU).

Immunohistochemistry and determination of MVD

Harvested preputial specimens were fixed in formalin, and then embedded in paraffin blocks. Then 5-μm-thick consecutive sections were deparaffinized and hydrated through a graded series of alcohol. Immunohistochemical staining was carried out with the avidin—biotin—peroxidase system using a monoclonal antibody (CD31/PECAM-1, clone JC/70A; LabVision Corp., Neomarkers, Fremont, CA, USA) against the pan-endothelial cell antigen CD31 (platelet/endothelial cell adhesion molecule). Microvessel areas were defined as vascular areas delineated by CD31-positive staining, and were identified in low-power (×100) fields using a light microscope (Olympus CX 41, Hamburg, Germany) by two pathologists blinded to the study 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 five random high-power (×200) fields (unit areas) within these hot spots. The mean results were recorded as MVD, and were expressed as blood vessels per unit area.

Statistical analysis

All statistical analysis was performed using SPSS software for Windows (release 17.0, SPSS Inc). All data were presented as mean ± standard deviation. All parameters were analyzed by Student's *t* test and Pearson's correlation analysis. The probability values less than 0.05 were considered statistically significant.

Results

The mean ages in the hypospadias and the control groups were 4.87 ± 2.30 and 6.10 ± 2.23 years, respectively. There was no statistically significant difference between the groups in terms of age ($p > 0.05$). The preputial MVDs and BFs of the study groups were shown in Fig. 1. The preputial MVD was found to be significantly decreased in the hypospadias group compared with the control group (33.95 ± 9.79 vs. 48.25 ± 10.08; $p < 0.05$), whereas there was no difference in terms of the BF (40.58 ± 16.16 vs. 33.09 ± 19.65; $p > 0.05$).

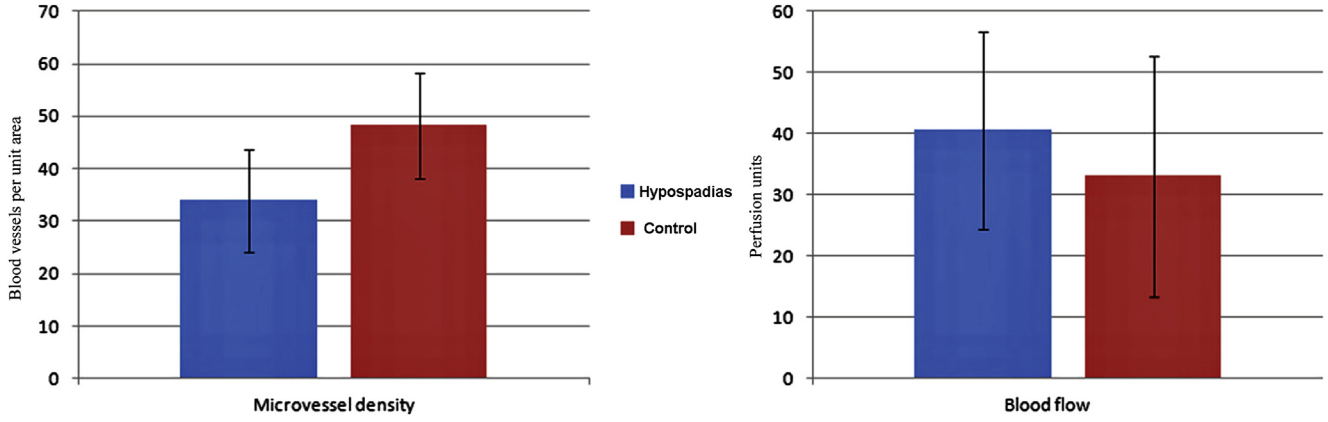

Figure 1 The microvessel density and blood flow values of the study groups.

Discussion

The prepuce is an ideal material for urethral reconstruction in hypospadias surgery. The main vascular supply of the normal prepuce derives from the superficial penile arteries, which arise from the inferior external pudental arteries. The braches course in the dartos facia, and become tortuous and minute on reaching the preputial ring, and the terminal branches are running circumferentially toward the coronal sulcus [3]. The arterial blood supply of the hypospadiac prepuce is different than normal. Therefore, better understanding of the preputial vascular anatomy may have an impact on the surgical outcome, where the preputial island flaps are used for the urethral reconstruction.

Several studies have examined the vascular anatomy of the hypospadiac prepuce. According to the anatomical studies of Hinman [4], the inner layer of the prepuce was ideal for urethroplasty without jeopardizing the vascular pedicle. Perovic and Radojicic [5] investigated the vascular anatomy of hypospadiac prepuce using preputial illumination, and concluded that a prepuce with a net-like vascularization without predominant artery was unsuitable for preparing well-vascularized preputial flaps for urethral reconstruction. Another study showed a significant correlation between morphology and vascularization of the prepuce, and suggested that underdeveloped prepuces and those with an unfavorable vascular pattern used for urethroplasty had a higher percentage of complications [6]. Yucel et al. [3] observed that the types of preputial vascular anatomy were similar between normal and hypospadiac males, whereas the frequency of each variation type was different. Particularly, the net-like arterial system (no predominant artery) was found more commonly in the hypospadiac prepuce. It was noted as an interesting finding that as the severity of hypospadias increased, the net-like vascularization became more common. Another study propounded that not only the vascular anatomy but also angiogenesis may be defective in hypospadiac prepuces [7]. More recently, Çağrı Savaş et al. [1] have demonstrated a defect in the MVD of hypospadiac prepuce. We herein found a similar significant decrease in the MVD (Fig. 2). All of mentioned studies are highly suggestive for a defective vascular pattern in hypospadiac prepuce. Nevertheless, data on the BF of hypospadiac prepuce are limited. In this respect, the present study adds some new information to the literature.

LDF is a diagnostic method of the continuous and non-invasive measuring of the microcirculatory BF in the different tissues. The technique is based on measurement of Doppler shift formed in the frequency of radiation that is reflected by the moving red blood cells. Currently the LDF is used in the areas of dermatology, dental and ocular applications, cardiovascular and transplant surgeries [2,8—12]. LDF has been also used to examine the viability of parameatal-based foreskin flap for urethroplasty in hypospadias repair [13]. We used this new diagnostic method to measure the BF of hypospadiac prepuce, and investigated whether defective vascular pattern in the hypospadiac prepuce correlates a decrease in the BF. Eventually, we found no correlation between the preputial MVD and BF in patients with distal hypospadias. But, we do not know what is the situation in proximal cases, where the preputial island flaps are used for the urethral reconstruction. The underlying cause leading to more severe hypospadias affects preputial development and its vascularization more commonly [1,3]. With this knowledge, we can speculate that patients with more severe hypospadias may have decreased preputial BF. Moreover, if preputial BF shows a parallel decrease with the MVD in the proximal cases, LDF may be used as a non-invasive diagnostic method to identify preoperative vascularization of the hypospadiac prepuce. Thus, we can decide which patient will receive preoperative testosterone treatment, where it could have beneficial effects in the preputial island flap urethroplasty. Because recent studies showed that testosterone increases vascularity of the hypospadiac prepuce [14—16].

In conclusion, the results of the present study are supportive of previous studies suggesting a defective vascular pattern in the hypospadiac prepuce. But, the BF of the hypospadiac prepuce remains unaffected despite this defective vascular pattern. Nevertheless, we realize the limitations of the present study. Statistical power may have not been sufficient to detect a significant difference in BF due to a small number of the patients. Additionally, our study is limited with only distal cases because of the

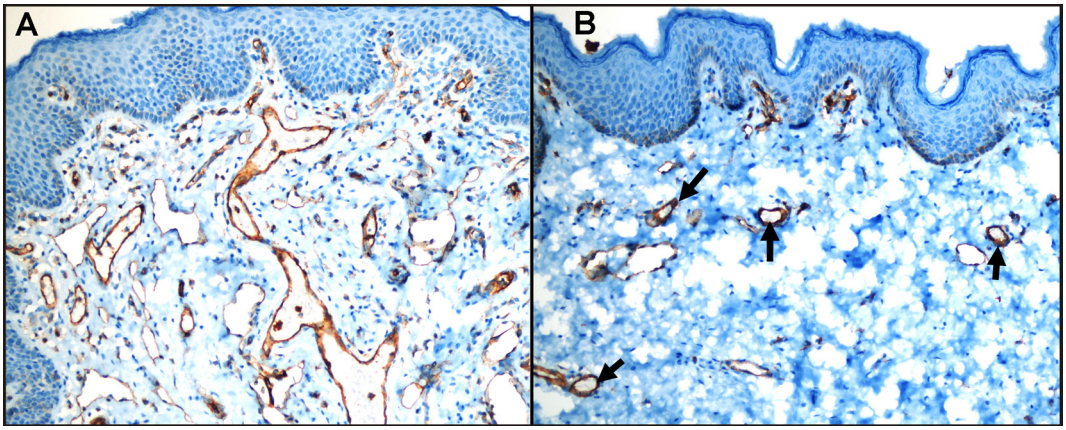

Figure 2 The microscopic appearance of the microvessels (immunohistochemical CD31 staining, 200×). (A) Normal preputial microvessels in the control group. (B) Arrows show the decreased preputial microvessels in the hypospadias group.

relatively low incidence of proximal hypospadias in our city. Patients with hypospadias may have decreased preputial BF, but further studies including larger numbers of patients with severe hypospadias are needed to verify this result.

Conflict of interests

None.

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