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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; $p > 0.05$).

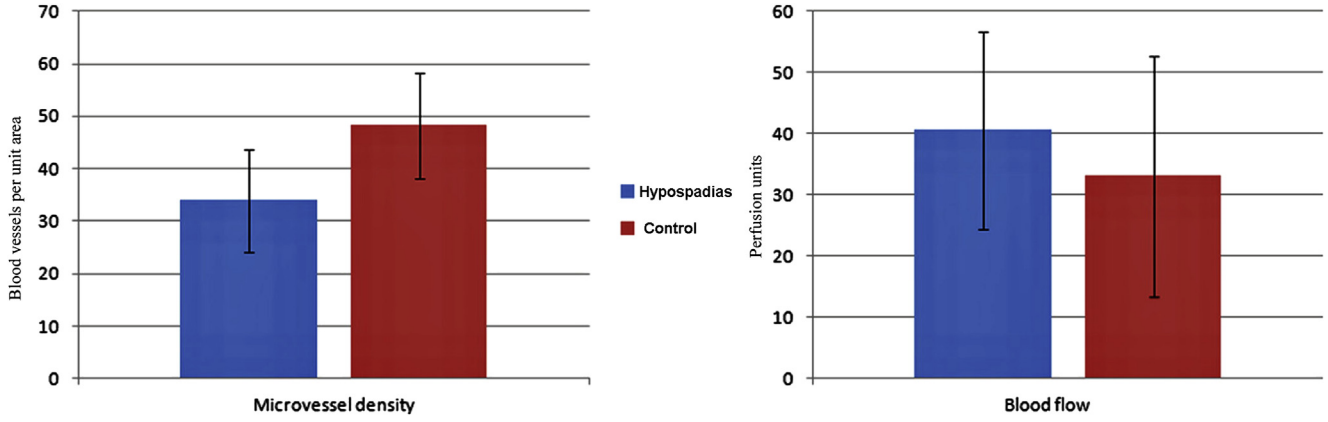

Figure 1 The microvessel density and blood flow values of the study groups.