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The microvessel density of the hypospadiac prepuce in children☆

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Abstract *Objective:* The blood supply of the hypospadiac prepuce is crucial for success in surgery. Although the vascular anatomy of the hypospadiac prepuce has been previously documented, data on microvessel density are missing. It was aimed to document the microvessel density of the normal and hypospadiac prepuce.
Methods: After ethical approval of the study, prepuces from 24 children with hypospadias undergoing surgical repair and from 9 healthy age-matched controls were stained by immunohistochemical methods using panendothelial cell antigen CD31 in order to assay their microvessel density.
Results: The microvessel density was significantly decreased in hypospadiac children when compared to controls ($P < 0.05$). A gradual decrease in microvessel density was observed as the severity of the condition increased, indicating a significant negative correlation ($r = -0.585$; $P < 0.05$).
Conclusion: Our findings should be taken into account when considering preoperative treatments such as topical testosterone application or operative strategies for hypospadias using prepucial flaps, and also during the postoperative course, especially when it is complicated. Further studies are needed to clarify the role of vascularity in the pathogenesis of hypospadias and its consequences for surgical repair.

Introduction

Hypospadias is the most common congenital anomaly of the penis and its incidence has doubled since the 1960s [1]. It is defined by abortive development of the urethral spongiosum and ventral prepuce along with an arrest in the normal embryological correction of penile curvature [2].

The blood supply of the hypospadiac prepuce is crucial for successful hypospadias surgery, because it can be used for neourethra reconstruction and penile body skin closure. The vascular anatomy of the prepuce is also proposed as an important factor when choosing the operative technique, and may have an influence on the surgical outcome. A better knowledge of the vascular anatomy of the

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hypospadiac prepuce may improve the surgical results of hypospadias repair [3].

The morphology and vascular anatomy of the prepuce have been previously documented in both normal and hypospadiac subjects [4,5]. However, to the best of our knowledge, the microvessel density of hypospadiac prepuce has never been investigated before. This study was conducted in order to compare the microvessel densities of normal and hypospadiac prepuces by means of immunohistochemical methods using the panendothelial cell antigen CD31.

Patients and methods

Permission from the local ethics committee was obtained (IRB approval: 0863-M-04), and the use of redundant prepuce in the study was explained to the parents at the time of their informed consent. Thirty-three children who presented to the outpatient department for circumcision (9/33) or hypospadias operation (24/33) were included in the study. The same surgeon operated on all the patients, who were under general anesthesia. All patients with hypospadias were undergoing their first surgical procedure, and none of the control subjects had suffered an attack of balanitis before circumcision. Hypospadiac patients who had testosterone treatment prior to surgery were excluded from the study.

Study design

The most distal part of the outer preputial layer from the dorsal side of the penis was harvested for histological evaluation. Paraffin-embedded prepuce samples from 33 children were evaluated in this study. The prepuces from nine healthy children, aged 1—10 years (mean 5.44 ± 3.20), undergoing routine circumcision served as controls. The prepuces of 24 age-matched children between 1 and 12 years (mean 6.16 ± 3.15) undergoing hypospadias repair served as the study group. These 24 children were further divided into four subgroups, depending on the location of their urethral opening, as follows: glanular ($n = 8$), coronal ($n = 7$), subcoronal ($n = 5$), and penile ($n = 4$).

Immunohistochemistry

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 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 inhibition of endogenous peroxidase activity by immersion in 3% H_2O_2/methanol solution, antigen retrieval was conducted using 10 mmol/L citrate buffer (pH 6.0) in a microwave oven for 10 min at 120 °C. Sections were incubated with primary antibodies, thoroughly washed in phosphate-buffered saline, then incubated with biotinylated secondary antibody, followed by the avidin—horseradish peroxidase complex (LabVision). Finally, immune complexes were visualized by incubation with DAB chromogen (LabVision), 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 negative controls showed low background staining (data not shown).

Determination of microvessel density

Prepuce microvessels included capillaries and small venules and arterioles. When measuring microvessel density, 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 the CD31-stained vessels. They were identified as transversally sectioned tubes with a single layer of endothelial cells, either without or with a thin basement membrane. Using a light microscope (Olympus CX41, Hamburg, Germany), areas of high-density staining were identified in low-power (×100) fields by two pathologists 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 five random high-power (×200) fields within these hot spots. The mean results were recorded for analysis.

Statistics

All data are reported as means ± 1SD. Data were analyzed by SPSS for Windows (version 15.0, SPSS Inc.). 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 pair-wise comparisons. Correlation analysis between microvessel density and severity of hypospadias was done using the Spearman test. All tests were two sided and the probability values less than 0.05 were considered statistically significant.

Results

The mean microvessel density of control prepuces was 79.71 ± 20.03 and significantly decreased to 48.58 ± 25.69

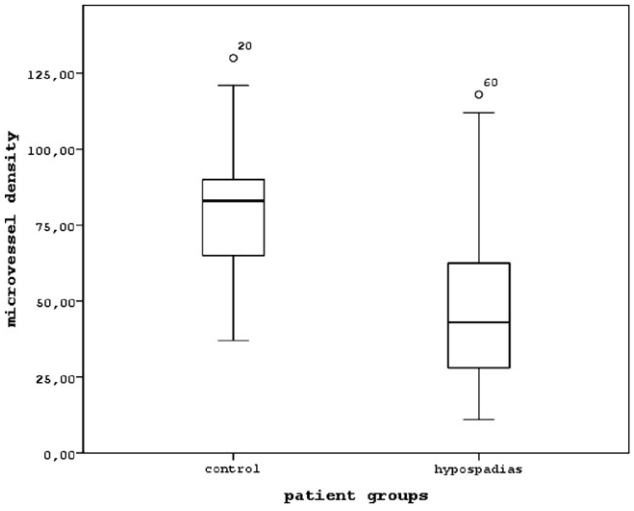

Figure 1 Microvessel density values of control and hypospadias patients in box plot.