Journal of the Anatomical Society of India 66 (2017) 91–96

Contents lists available at ScienceDirect

Journal of the Anatomical Society of India

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

Original Article

Nerves in the cavernous tissue of the glans penis: An immunohistochemical study using elderly donated cadavers

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ARTICLE INFO

Article history:
Received 3 April 2017
Accepted 25 November 2017
Available online 26 November 2017

Keywords:
Glans penis
Corpus cavernosum
Nerve supply
Neuronal nitric oxide synthase
Tyrosine hydroxylase

ABSTRACT

Introduction: The topographical anatomy of the composite nerve fibers in the human glans penis is poorly characterized. Therefore, histological methods were used to analyze nerves to the cavernous tissue at the distal end of the corpus spongiosum.
Methods: Immunohistochemical techniques were used to detect S100, neuronal nitric oxide synthase, tyrosine hydroxylase, and vasoactive intestinal polypeptide protein expressions in frontal or sagittal penile sections obtained from 20 donated, older male cadavers.
Results: At or near the coronal sulcus at the dorsal midline, three to seven terminal branches of the unilateral dorsal nerve ran deeply or centrally along the distal dull end of the corpus cavernosum and entered the glans cavernous tissue. Once there, the nerve divided into thinner branches (neuronal nitric oxide synthase or tyrosine hydroxylase positive) and the major nerve section reached the surface skin of the glans. Several thin nerves took highly arduous paths, as evidenced in ventral subcutaneous tissue of the distal third of the penis.
Discussion: Histological examination revealed a neurovascular bundle that penetrated the glans cavernous tissue toward the skin covering, with a rich nerve supply to the skin folds at and near the coronal sulcus.

1. Introduction

The dorsal nerve of the penis transmits sensory innervations from the glans penis and foreskin, and plays role in ejaculatory reflexes via somatosensory input from the glans.[1] Lateral branching provides afferent fibers to the corpus spongiosum and urethral mucosa.[2] A recent macroscopic study has shown that its terminal branches pass the subcutaneous course.[3]

However, immunological studies showing how the nerves innervate the human glans penis have not been performed. There is very little information about the nerve supply to the glans penis cavernous tissue or the distal end of the corpus spongiosum, although the nerve supply to the corpus cavernous penis at the penile hilum has been studied.[4,5] Consequently, the initial aim of this study was to describe the nerve histology in the human glans penis.

Previous studies using surgically obtained, but diseased, human penises have provided information about the immunohistological and chemical coding of the dorsal nerve of the penis.[6–9] However, immunohistological evaluation of the glans penis in normal adult human cadavers has not yet been reported. Therefore, the second aim of the study was to examine the immunohistochemical coding of the nerves to the glans penis.

2. Materials and methods

The study was performed in accordance with the provisions of the Declaration of Helsinki.[10] We examined 20 donated male

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https://doi.org/10.1016/j.jasi.2017.11.001
0003-2778/

cadavers ranging in age from 69 to 79 years, with a mean age of 73 years. The causes of death were ischemic heart failure or intracranial bleeding. We verified that none of the individuals had a history of surgery, including circumcision. Additionally, we performed macroscopic examinations for surgical scars in the abdominopelvic cavity. The cadavers were donated to the Tokyo Dental College for research and education on human anatomy, and their use for research was approved by the university ethics committee. The donated cadavers were fixed by arterial perfusion with 10% v/v formalin solution and stored in 50% v/v ethanol solution for more than 3 months. We prepared sagittal macro slices (10–12 mm thick) of the penis in 16 of the 20 cadavers, while the remaining four were used for frontal sections. A total of four macro slices were made from each of the 20 specimens. After performing routine procedures for paraffin-embedded histology, large sections (70×50 mm; 15 μm thick) were prepared at 1-mm intervals from each of the slices. After observations of the large sections, we cut sections for immunohistochemistry near the former large sections (70×25 mm). Thus, from a single paraffin block containing a 10-mm-thick macro slice, we prepared 10 large sections and 50 sections of normal size.

The large sections were stained with hematoxylin and eosin or Masson trichrome staining, while the normal size sections were used for immunohistochemistry. The primary antibodies for immunohistochemistry were as follows: mouse monoclonal anti-human S100 protein (1:100; Dako Z0311; Dako, Glostrup, Denmark); rabbit polyclonal anti-human nitric oxide synthase (nNOS) (1:100; Cell Signaling Technology, Beverly, MA, USA); mouse monoclonal anti-human vasoactive intestinal peptide (VIP) (1:100; sc25347; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA); and rabbit polyclonal anti-human tyrosine hydroxylase (TH) (1:100; ab152; Millipore-Chemicon, Temecula, CA, USA). According to our experience, S100 protein is the best marker for longer preserved cadaveric specimens, rather than PGP9.5, a neuron-specific enolase, or PMP22.[11] In the present study, we examined distribution of autonomic nerves using S100 protein as a pan-neuronal marker, TH as a sympathetic nerve marker, nNOS and VIP as a parasympathetic nerve marker. The secondary antibodies were labeled with horseradish peroxidase (HRP), and antigen-antibody

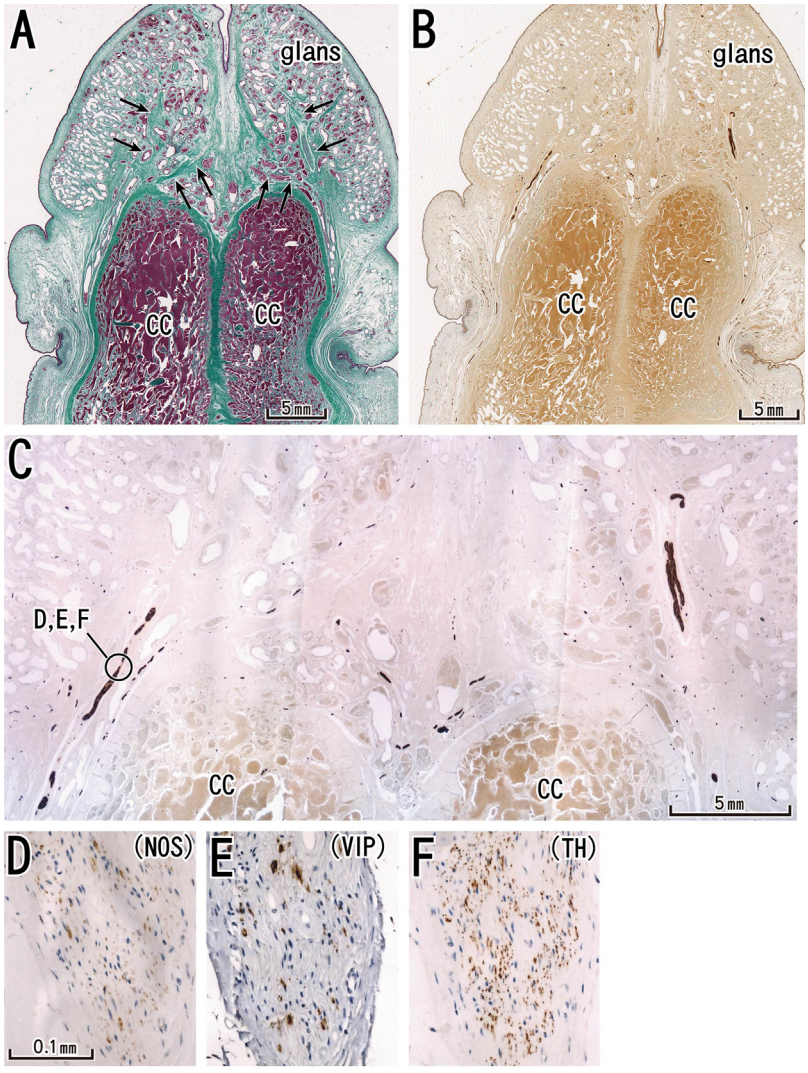

Fig. 1. Frontal sections of nerves to the glans penis from a 69-year-old man.
Panel A (Masson trichrome staining) and panel B (immunohistochemistry of S100 protein) are from adjacent sections across the dorsal end of the navicular fossa. Arrows in panel A indicate vessels perforating the glans. Panel C is a higher magnification view of the center of panel B. Multiple terminal branches of the dorsal nerve of the penis run along the distal end of the corpus cavernosum (CC) to show a centrally shifted course. Panels D (immunohistochemistry of neuronal nitric oxide synthase, nNOS), E (vasoactive intestinal polypeptide, VIP), and F (tyrosine hydroxylase, TH) show nerve fibers, as indicated by a circle in panel C. Panels D–F are from the same magnification (scale bar in panel D).

reactions were detected by the HRP-catalyzed reaction with diaminobenzidine. Hematoxylin counterstaining was performed on the same samples. A negative control without the primary antibody was set up for each specimen. Observations and images were collected with a Nikon Eclipse 80 microscope (Nikon, Tokyo, Japan). Images at ultralow magnification ($< \times 1$ objective) were collected using a high-grade flat scanner with a translucent illumination scanner (GTX970; Epson, Tokyo, Japan).

We measured density of S100-positive nerves inside the 1-mm squares. In each specimen, we chose two hotspots to quantify the largest number of nerves in the squares according to the previously described hotspot method of Weidner et al. to quantify vessel density.[12]

All analyses were performed using JMP® (Version 10.0, SAS Institute, Cary, NC, USA). The Wilcoxon rank sum tests were performed on all data sets, as well as for nonparametric data. Results represent mean ± SD, with $P < 0.05$ considered statistically significant.

3. Results

The large tissue sections covered a wide area that included the distal 1/3–1/2 of the penis (Figs. 1–3: these figures illustrate the glans penis, as well as the terminal or intra-glans courses of the dorsal nerve of the penis). At and near the coronal sulcus, in the final subcutaneous course, the dorsal nerve divided into abundant

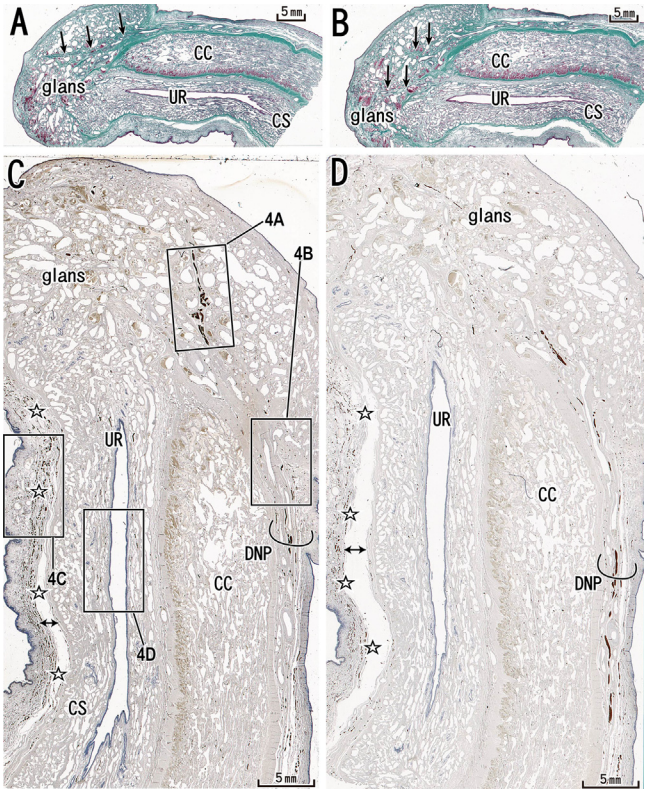

Fig. 2. Near mid-sagittal sections of the penis of a 74-year-old man.
Panels A and B (Masson trichrome staining) are separated by 2 mm. Arrows indicate vessels perforating the glans. Panels C and D (immunohistochemistry of S100 protein) are higher magnification views of the distal part of panels A and B, respectively. Terminal branches of the dorsal nerve of the penis (DNP) run along the distal end of the corpus cavernosum (CC) to show a ventrally shifted course. Two or three branches enter the cavernous tissue of the glans. Double-headed arrows (panels C and D) indicate a space artifact as a result of the histological procedure. The ventral subcutaneous tissue contains abundant wavy nerves at and near the coronal sulcus (stars). Panels A and B (C and D) are from the same magnification. CS, corpus spongiosum; UR, penile urethra.

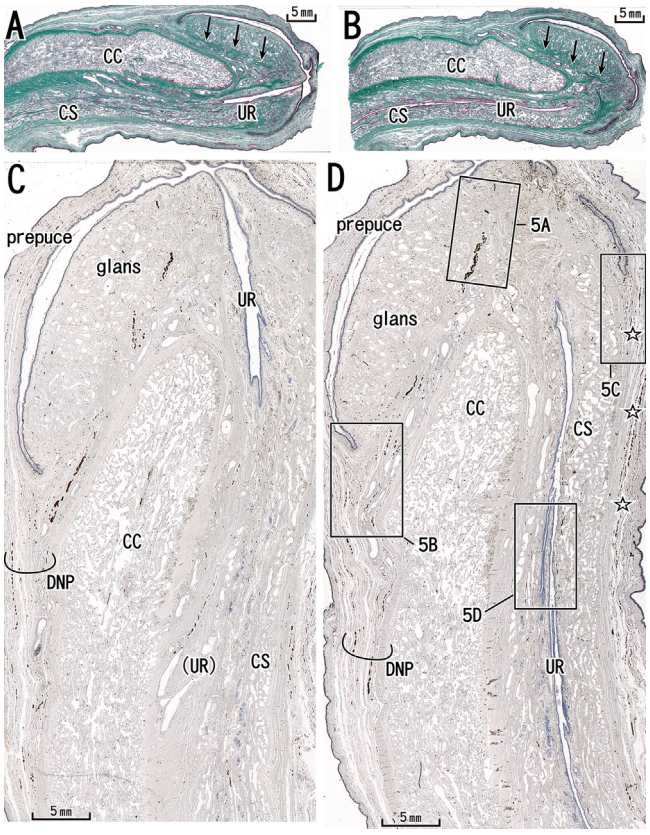

Fig. 3. Near mid-sagittal sections of the penis of a 75-year-old man.
Panel A and B (Masson trichrome staining) are separated by 2 mm. Arrows indicate vessels perforating the glans. Panels C and D (immunohistochemistry of S100 protein) are higher magnification views of the distal part of panels A and B, respectively. Terminal branches of the dorsal nerve of the penis (DNP) run along the distal end of the corpus cavernosum (CC) to show a ventrally shifted course. Multiple branches enter the cavernous tissue of the glans. The ventral subcutaneous tissue contains abundant wavy nerves (stars). Panels A and B are from the same magnification, as are panels C and D. CS, corpus spongiosum penis; UR, penile urethra.

branches that innervated the cavernous tissue at the base of the glans (i.e., corpus spongiosum) and the prepuce (Figs. 4 and 5). There were three to seven (median of five) relatively thick unilateral terminal branches (0.1–0.3 mm in diameter) that followed relatively straight pathways either ventrally or centrally (deeply) along the distal blunt end of the corpus cavernosum toward a tight space near the dorsal end of the navicular fossa. The thicker nerves did not enter the prepuce. The terminal branches consistently accompanied the artery and vein in and along the glans, although the blood vessels entering the glans were not usually accompanied by nerves. The neurovascular bundles in the glans were enclosed by thick, collagenous sheathes. Along the pathways at the base of the glans, the nerves formed multiple branches towards the cavernous tissue of the glans (Figs. 1 C, 4 A, and 5 A). Several of the nerves that followed more arduous paths communicated bilaterally across the midline along the dorsal aspect of the urethra or navicular fossa. Finally, most of the multiple terminal branches of the dorsal nerve reached the skin of the dorsal half of the glans, while some reached the ventral half of the skin and the navicular fossa. Conversely, because the thinner branches were greater in number, nerve density in the glans skin was statistically higher in the ventral half [5–12 (median 8) branches/1 mm2] than in the dorsal half [2–5 (median 3) branches/1 mm2] when the cut surfaces were quantified in the sagittal sections ($P < 0.01$).

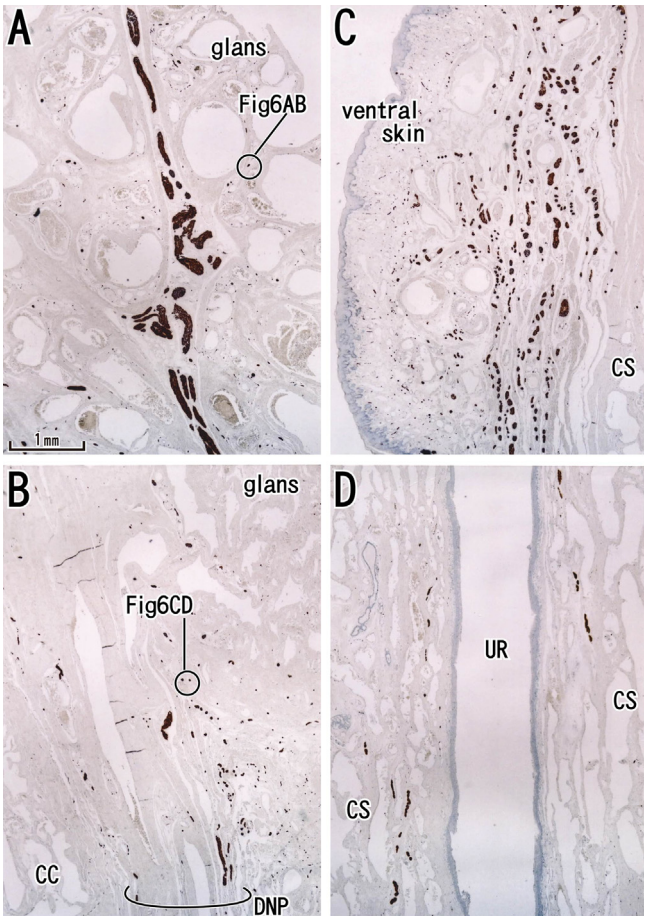

Fig. 4. Higher magnification view of nerves in the penis of a 74-year-old man. Immunohistochemistry of S100 protein. Panel A (terminal branches perforating the glans), panel B (dorsal nerves near the coronal sulcus), panel C (ventral subcutaneous nerves), and panel D (a few nerves along the urethra) correspond to squares from 4A, 4B, 4C, and 4D in Fig. 2C, respectively. The cavernous tissue of the glans contains fewer thin nerves in the distal site (panel A) than proximal site (panel B). All panels are from the same magnification (scale bar in panel A). CS, corpus spongiosum; DNP, dorsal nerve of penis; UR, urethra.

There were differences in nerve density between individuals in the cavernous tissue of the glans. For example, Fig. 3C (a 75-year-old man) shows greater numbers of nerve branches than in Fig. 2C (a 74-year-old man). In some specimens, there were fewer cavernous nerves inside the glans distally (Fig. 4A) than proximally (Fig. 4B). Along neural pathways at the base of the glans, the terminal branches issued multiple branches to cavernous tissue of the glans (Figs. 1 C, 4 A, and 5 A), which contained not only thick myelinated fibers, but also abundant thin nerve fibers that expressed nNOS, VIP, or TH (Figs. 1 D, E, and F, and 6). There were no differences in composition of nerve fiber types between individuals: TH-positive fibers were the dominant type in glans from all specimens. A total of two to three (median of two) nerves ran along the urethra, but we found no communication with the dorsal nerve in the distal 1/3 of the penis (Figs. 4 D and 5 D). The skin folds at and near the coronal sulcus contained abundant thin nerves, especially on the ventral side (Figs. 1 B, 2 C, and 3 D), and each of the nerves appeared as “beads” connected by a straight string as a result of the highly arduous pathway (Figs. 4 C and 5 C). The corpus cavernosum, which included the midline septum and the tunica albuginea, contained three to five (median of four) nerves running longitudinally. The dorsal nerve of the penis did not give off perforating branches into the tunica albuginea toward the distal part of the corpus cavernosum.

4. Discussion

Histological results obtained from human male cadavers in the present study are the first to demonstrate a terminal course for the dorsal nerve of the penis in the glans. The neurovascular bundle, with a relatively thick collagenous sheath, penetrates the cavernous tissue of the glans toward the overlying skin. Additionally, our results revealed a rich nerve supply to the skin folds at and near the coronal sulcus; the most striking feature was found in multiple longitudinal nerves that underwent arduous pathways along the ventral skin of the distal third of the penis.

Dorsal nerve courses and branches of the penis have been reported previously. Kozacioglu et al. demonstrated the macroanatomy of the dorsal nerve and found the presence of branches perforating the tunica albuginea.[3] Conversely, and based on our findings, perforating nerves did not branch off from the dorsal nerve and enter the tunica albuginea toward the distal part of the corpus cavernosum. Some nerves ran longitudinally along the urethra and in the corpus cavernosum. This finding was in contrast to previous reports,[3] which was likely owing to differences between macroscopic and histological observations. Macroscopic dissections of peripheral nerves from human cadavers are difficult and reproducibility is low, making it difficult to confirm results without histological verification. Therefore, immunohistochemical

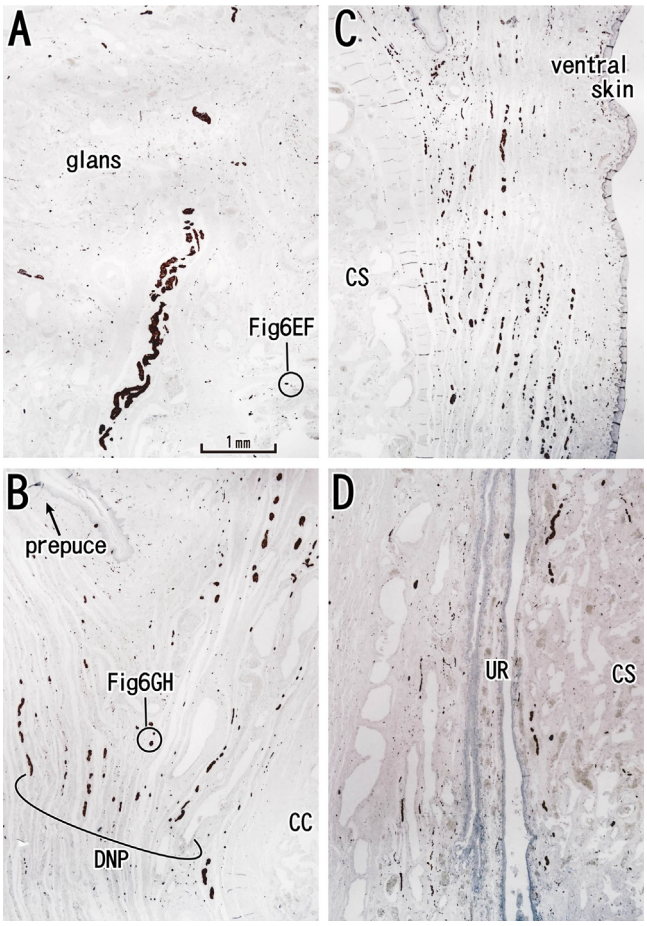

Fig. 5. Higher magnification view of nerves in the penis of a 75-year-old man. Immunohistochemistry of S100 protein. Panel A (terminal branches perforating the glans), panel B (dorsal nerves near the coronal sulcus), panel C (ventral subcutaneous nerves), and panel D (nerves along the urethra) correspond to squares from 5A, 5B, 5C, and 5D in Fig. 3D, respectively. The nerve density in the cavernous tissue of the glans (panel A) is greater than in Fig. 4A. All panels are from the same magnification (scale bar in panel A). CS, corpus spongiosum; DNP, dorsal nerve of penis; UR, urethra.

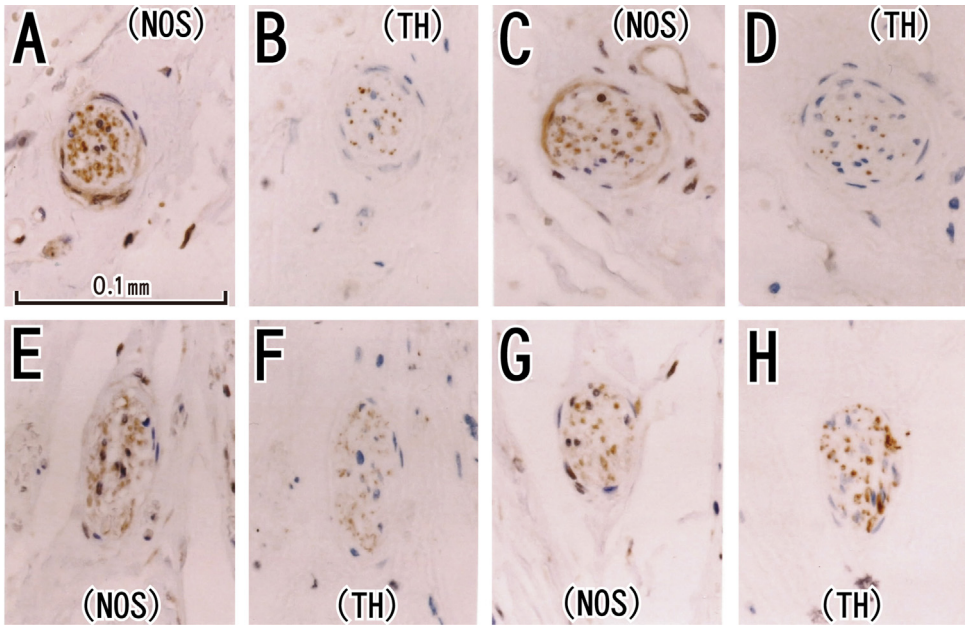

Fig. 6. Nerve fibers in the penis.
Immunohistochemistry of neuronal nitric oxide synthase (nNOS) and tyrosine hydroxylase (TH). Panels A and B (C and D) reveal a nerve from Fig. 4A (4B). Panels E and F (G and H) show a nerve from Fig. 5A (5B). All thin nerves equally contain TH-positive sympathetic and nNOS-positive parasympathetic nerve fibers. All panels are from the same magnification (scale bar in panel A).

staining and microscopic observation are mandatory for demonstrating the precise pathways of peripheral nerves.

Müller et al. showed that the corpora cavernosa and corpus spongiosum are mainly supplied with autonomic filaments, and the sensitive glans penis is principally supplied with sensory nerves.[13] They also reported that the greatest portion of dorsal nerve branches of the penis are anterior, spreading out partly into the foreskin, but especially into the glans penis where the end of the nerves enters in bunches. Results from the present study extended our knowledge from previous reports; immunohistochemistry revealed terminal nerve branches in the glans that contained not only thick myelinated fibers (candidate sensory fibers), but also abundant thin nerve fibers that expressed nNOS, VIP, or TH. The latter fibers have been shown to modulate corpus spongiosum function as an effective arteriovenous shunt.[14] Tamura et al. also reported on the coexistence of nNOS, TH, and VIP in surgically removed penile tissue.[6] To the best of our knowledge, the present study provides the first immunohistological confirmation of autonomic nerves from the corpus spongiosum (CS) and the corpus cavernosum (CC) in human male cadavers.

Differences in nerve density in the cavernous tissue of the glans exist between individuals. However, in the present study, increased age of the cadaver did not correlate with decreased numbers of composite nerve fibers in the human glans penis or nerves to the cavernous tissue. Therefore, this difference might be attributed to a difference in nerve immunoreactivity.

Circumcised men are reported to show significantly lower levels of sexual pleasure sensation of the glans, especially at the dorsal site of the glans,[15] although a definitive conclusion has not yet been reached. This loss of sensation has been explained by the resected prepuce, which contains specialized sensory mucosa,[16] or the frenulum, which contains high-density nerve content.[17] Circumcision does not induce injury to nerves that innervate the glans penis, including the sulcus, because circumcision is a dermal operation. However, incision into tissues beneath the dermis can occur by inappropriate techniques.

The majority of the dorsal nerve branches in the penis terminate in the skin of the dorsal half of the glans, although some terminate in the ventral half of the skin. However, the ventral half is divided into many, thinner branches, thus nerve density is greater in the ventral half than in the dorsal half of the glans skin. Thus, careless surgical incisions of the ventral skin could cause sensory nerve damage because of the high nerve density. Because there are few communicating branches across the midline at the base of the glans, nerve regeneration from the contralateral side would be unlikely after unilateral nerve damage.

Cavernous nerves, arising from the pelvic plexus, are composed of parasympathetic fibers from the second, third, and fourth sacral spinal nerve, and they run parallel to the corpora cavernosa artery and facilitate penile erection.[13] Results from the present study show the pathways and terminus of the cavernous nerve in the glans penis. However, whether autonomic nerves of the glans penis affect erection remains to be determined. Nevertheless, special care should be taken during surgical procedures to avoid injury to nerve fibers in the glans penis.

The present study has some limitations. First, cooperative action between nerves and vasodilation was not elucidated in this study. Second, the mean age of the cadavers could affect the composite fibers of nerves in the human glans penis, as well as nerves to the cavernous tissue. Nevertheless, this study does provide a valuable reference for further investigations into the role of these nerves in the glans penis and new insights into the structure and function of the human glans penis.

5. Conclusions

Histological analysis revealed that the neurovascular bundle penetrates the cavernous tissue of the glans toward the overlying skin, and a rich nerve supply to the skin folds exists at and near the coronal sulcus. The anatomical details of the glans penis described in the present study provide an anatomical background for penile surgeries, especially for making fine adjustment in incision trajectories.

Financial Disclosure

The authors declare that they have no relevant financial interests.

Conflict of Interest

No conflicts of interest

Acknowledgment

This study was supported by Wonkwang University in 2017.

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