Redefining the Autonomic Nerve Distribution of the Bladder Using 3-Dimensional Image Reconstruction

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Purpose: We sought to create a 3-dimensional reconstruction of the autonomic nervous tissue innervating the bladder using male and female cadaver histopathology.

Materials and Methods: We obtained bladder tissue from a male and a female cadaver. Axial cross sections of the bladder were generated at 3 to 5 mm intervals and stained with S100 protein. We recorded the distance between autonomic nerves and bladder mucosa. We manually demarcated nerve tracings using ImageScope software (Aperio, Vista, California), which we imported into Blender™ graphics software to generate 3-dimensional reconstructions of autonomic nerve anatomy.

Results: Mean nerve density ranged from 0.099 to 0.602 and 0.012 to 0.383 nerves per mm^2 in female and male slides, respectively. The highest concentrations of autonomic innervation were located in the posterior aspect of the bladder neck in the female specimen and in the posterior region of the prostatic urethra in the male specimen. Nerve density at all levels of the proximal urethra and bladder neck was significantly higher in posterior vs anterior regions in female specimens (0.957 vs 0.169 nerves per mm^2, $p < 0.001$) and male specimens (0.509 vs 0.206 nerves per mm^2, $p = 0.04$).

Conclusions: Novel 3-dimensional reconstruction of the bladder is feasible and may help redefine our understanding of human bladder innervation. Autonomic innervation of the bladder is highly focused in the posterior aspect of the proximal urethra and bladder neck in male and female bladders.

Key Words: urinary bladder; urethra; models, anatomic; autonomic pathways; cadaver

Abbreviations and Acronyms

3D = 3-dimensional

OAB = overactive bladder

UUI = urge urinary incontinence

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Overactive bladder is a common health problem worldwide with a significant impact on patient quality of life. In the United States OAB affects 37 million adults and is responsible for more than $12 billion in health care costs annually.[1,2] Additionally many patients with OAB experience UUI and require medical or invasive therapies to manage lower urinary tract symptoms.

The etiology of OAB and UUI is unknown in the majority of patients.[3,4] Treatment of OAB is generally directed at decreasing abnormal contractions of detrusor muscles, and targeting urothelial and afferent nervous tissue by medical or surgical

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intervention. In addition to several effective anticholinergic medications, invasive therapies such as neuromodulation and botulinum injections have been developed in recent years to reduce urgency and incontinence symptoms in patients with OAB.[5–8] Despite these advances diagnosis and treatment remain challenging for many patients with idiopathic OAB. More precise understanding of the neural distribution of the bladder may lead to further improvements in neuromodulation, denervation or botulinum therapies for OAB.

Modern advances in computer aided image reconstruction technology allow microscopic nerve tissue in human organs to be systematically mapped in a 3D format.[9] A virtual 3D map of the autonomic nerve distribution of the bladder could redefine our understanding of the relationship between neural tissue and bladder urothelium. Furthermore, improved understanding of local neural anatomy could help improve targeted nerve modulating therapies and prevent unwarranted tissue damage during invasive procedures. Currently there is no comprehensive model that provides a detailed 3D view of bladder autonomic innervation. Thus, we developed a 3D image reconstruction of autonomic nerve tissue innervating the urethra, bladder neck and bladder using male and female cadaver histopathology.

MATERIALS AND METHODS

Anatomical Dissection

We obtained approval from the Willed Body Program at University of California-Irvine to use 2 donated human cadavers and performed anatomical pelvic dissections on 1 male and 1 female. The female was in the eighth decade of life and died of advanced dementia related to cerebral vascular disease. The male was in the ninth decade of life and died of respiratory failure. Neither cadaver had a known history of malignancy, UUI, benign prostatic hyperplasia or any other urological condition. We procured urethra and bladder specimens intact with adjacent organs, including the prostate and seminal vesicles in the male, and the vagina, cervix and uterus in the female.

To evaluate a semidistended bladder we filled the bladder lumen with paraffin via a Foley catheter and the paraffin was allowed to cool and solidify. We then performed transverse sections beginning at the distal urethra and ending at the bladder dome. Portions of the bladder specimen with a large circumference were cut in half sagittally before transverse sectioning. The transverse sections were made 3 mm apart in the male and 5 mm apart in the female, and placed on slides for histology.

Histological Evaluation

We prepared the histological sections by baking at 60C for 30 minutes. Paraffin was removed by 3 wash cycles of xylene. Sections were rehydrated through a series of washes with 100% alcohol, 95% alcohol and purified water. We performed endoperoxidase block in 70% methanol/50% hydrogen peroxide solution for 10 minutes. After thorough rinsing with purified water we pretreated the slides using citrate buffer (pH 6.0) for 25 minutes at 90C in a vegetable steamer. We then incubated the slides for 45 minutes at room temperature with rabbit S100 antibody (No. Z0311, Dako North America, Carpinteria, California) diluted 1:600 with casein. This was followed by rinsing with phosphate buffered saline-Tween® and incubation with EnVision® System Labelled Polymer HRP anti-rabbit secondary antibody (No. 4003) for 30 minutes at room temperature. We again rinsed the slides with phosphate buffered saline-Tween. Anti-rabbit antibodies were detected with a Betazoid DAB Chromogen Kit (No. BDB2004L, Biocare Medical, Concord, California). Finally the sections were rinsed with water, counterstained with hematoxylin, dehydrated through graded ethanol, cleared with xylene and coverslipped.

Autonomic Nerve Distribution

We obtained histology images from male and female bladder specimens. We reapproximated halved histology slides using high resolution ImageScope software. We visually identified autonomic nerve tissue by a positive S100 stain. We then manually demarcated each nerve site contained in anatomical structures of the urinary tract, including the bladder detrusor muscle, bladder neck and proximal urethra. Nerves surrounding urological organs or contained in adjacent organs such as the vagina were not evaluated. We uploaded histology images to AutoCAD® computer aided design software. Each slide was divided into posterior, left, right and anterior quadrants based on anatomical orientation. For each histology slide we calculated the number of demarcated nerve sites and the areas of each quadrant using AutoCAD. Nerve density was calculated by dividing the number of nerve sites by the corresponding histology slide area. Finally we measured the distance of each nerve to the urothelial surface of the urethra or bladder using AutoCAD.

Three-Dimensional Image Reconstruction

We imported high resolution histology images into Blender 3D modeling and animation software. Serial 2-dimensional slides were aligned and appropriately spaced at 3 or 5 mm intervals for the male and the female bladder, respectively. We created cylinder shaped tracings to connect demarcated autonomic nerves between adjacent histological slides. These nerve tracings were labeled in yellow. Similarly we traced the demarcated borders of urethral, bladder and vaginal epithelia to create a virtual 3D reconstruction of the urethra, bladder and vagina, respectively. Using Blender software we rendered the 3D model of autonomic nerve distribution into a high resolution image. Finally we constructed a color coded map of the autonomic nerve distribution to the bladder using Blender software to visually represent calculated values of nerve density.

RESULTS

Histological Evaluation

In the male cadaver a total of 1,425 sites of autonomic nerve tissue were manually demarcated in 13