A Three Dimensional Nerve Map of Human Bladder Trigone

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Aim: Central efferent and afferent neural pathways to and from the human urinary bladder are well-characterized, but the location and arborization of these nerves as they traverse the serosa, muscularis, and urothelial layers are not clearly defined. The purpose of this study was to create a three-dimensional map of the innervation of the human bladder trigone from the extrinsic perivesical adventitial nerve trunks to the urothelium. Methods: A male and a female human bladder were harvested from fresh frozen cadavers and fixed in formalin. The bladder neck and trigone region were serially sectioned (5 μm) and every 20th slide was stained (S100), scanned and aligned to create 3D maps. Results: Nerve penetration into the detrusor muscle occurs with the highest frequency at the bladder neck and interureteric ridge. Nerves traveling parallel to the bladder lumen do so in the adventitia, beyond the outer border of detrusor. In females, the depth of these nerve bands is uniform at 0.7–1.7 cm below the luminal surface, the outer limits of which include the anterior vaginal wall. In the male, depth is more variable owing to detrusor hypertrophy with the minimum depth of nerves approximately 0.5 cm near the interureteric ridge and over 1 cm near the bladder neck. Conclusions: Myelinated neural pathways traversing in the human bladder in the region of the trigone have a discreet regional density. This 3D map of trigonal innervation may provide guidance to more precisely direct therapies for urinary incontinence or pelvic pain. Neurourol. Urodyn. © 2016 Wiley Periodicals, Inc.

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INTRODUCTION

Knowledge of the innervation of the bladder is important to surgeons planning neuromodulatory, neuroablative, or nerve-sparing reconstructive procedures. While the nerve pathways that innervate the bladder are well-defined as they travel between the spinal cord and deep pelvis, their course as they traverse the serosa, muscularis, and urothelial layers are not.

Studies from the 1960s2 and 1970s3 described the distribution of nerves in the bladder itself, demonstrating that the trigone and bladder neck contained higher levels of adrenergic and muscarinic innervation compared to the body of the bladder. More recently, Spradling et al. assembled histochemical stains of myelinated nerves into a three-dimensional (3D) map of the whole human bladder.4 Their data confirmed that innervation is concentrated in the posterior bladder neck, extending into the trigone, and they propose that it is in this region that chemical denervation procedures should be targeted. The purpose of this study is to create a 3D map of human bladder innervation in this specific region with a focus on locating nerves as they penetrate from the adventitia through the bladder wall. This high resolution spatial imaging may be of significant importance in designing templates for chemo-denervation techniques as well as planning reconstructive surgery of the lower urinary tract.

Innervation to the bladder is complex and is derived from sympathetic and parasympathetic fibers with a contribution from somatic fibers that are responsible for providing volitional control of the external sphincter.7 The sympathetic fibers originating from spinal cord segments T10-L2 and parasympathetic fibers originating from S2-4 take their individual pathways to meet in the pelvic plexus. In relation to the ureterovesical junction, the pelvic plexus is located approximately 1.5–2 cm dorsomedially where it further branches in a manner that is gender dependent. In males, there are branches that travel to the seminal vesicles and prostate while females demonstrate branches that travel to the vagina and uterus. The majority of fibers, however, innervate the bladder by traveling along two possible pathways. Yucel and Baskin, along with other researchers, have shown that one set of nerve fibers travels to the distal ureter and then courses down the medial aspect of the ureter in the adventitia.5–7 A second group of fibers leave the pelvic plexus and terminate directly on the bladder trigone. Exactly where, and at what depth, the nerve fibers traverse the anatomical layers of the bladder has not been elucidated and is the focus of this investigation.

In this investigation, we harvested cadaveric tissue including the bladder, distal ureters, and the pelvic nerve plexuses en
bloc. Serial sectioning, immunohistochemical analysis, and three-dimensional reconstruction software has allowed us to construct a 3D map of innervation to the human trigone and the immediately surrounding area.

MATERIALS AND METHODS

Human Cadaveric Pelvis

Approval for this project as exempt research was obtained from the Institutional Review Board at the Medical University of South Carolina. One female and one male fresh frozen human pelves were obtained from Anatomy Gifts Registry (Hanover, MD) and thawed at 4°C in the morgue for 3–5 days until sufficient for dissection. The female passed away in her ninth decade from heart failure and the male was deceased in his eighth decade from ischemic bowel disease. Neither had a history of urological surgery nor benign or malignant urological pathology. Reception, storage, and dissection of the specimens were performed at the Center for Anatomical Studies and Education at the Medical University of South Carolina.

Harvest of Cadaveric Human Bladders

Through a vertical midline incision, the anterior surface of the bladder was exposed and the ureters were each identified above the crossing of the iliac vessels. The paravesical and pararectal spaces were defined and the visceral peritoneum above the crossing of the iliac vessels. The paravesical and pararectal spaces were defined and the visceral peritoneum was opened at the Pouch of Douglas in females and the vesicouterine fold in the male. The plane of dissection was followed along to the anterior rectal wall and care was taken not to disrupt the pelvic plexuses located approximately 1.5–2 cm dorsomedial to the ureters. The bladder was exposed and the ureters were each identified. They were then washed in PBS and blocked for 30 min in normal goat serum provided by the Vectastain ABC Staining kit (Vector Laboratories, Burlingame, CA). The slides were then incubated in the Polyconal Rabbit anti-S100 primary antibody (Dako, Carpinteria, CA) (1:500 dilution) for 1 hr. The slides were then washed in PBS and incubated in biotinylated secondary antibody (Vectastain) for 30 min. Following an additional wash, the slides were incubated for 30 min in the Vectorstain ABC Reagent. The slides were then washed and incubated in 3,3’ diaminobenzidine peroxidase substrate solution and developed for 10 min. Slides were covered, dried for >24 hr. and scanned as TIFF files at 10× magnification. Specimen thickness was measured with calipers.

Generation of 3D Bladder Nerve Maps

The histological sections were stacked and aligned in Adobe Photoshop (Adobe Systems, Inc., San Jose, CA) to recapitulate the whole specimen with clearly defined boundaries. The slides were then imported as stacked files into Amira software (FEI Visualization Sciences Group, Burlington, MA) and the surfaces were outlined, interpolated, and smoothed to create the 3D representation of the tissues. Individual nerves were identified in each section and the filament editor tool in the Amira software was used to interpolate the nerve pathways between each section and the 3D nerve map which was overlaid with the tissue model. Each ureteral orifice was identified histologically.

RESULTS

3D Map of Bladder Innervation in the Human Female

Figure 2 shows an AP image of the map demonstrating the 3D neural network in the human female bladder. The S100-stained nerves are colored red in this model, the detrusor is blue, and adventitia/anterior vaginal wall are aquamarine. The ureters are seen as yellow dots. From this view, as the bladder is split anteriorly and unfolded, one can appreciate the density of nerves that are most apparent at the 3 o’clock and 9 o’clock positions on the bladder neck just above the junction with the urethra. From each of these two bundles, there is a hand of nerves tracking between the bladder neck and the interureteric ridge. We then see significant nerve populations across the interureteric ridge, although somewhat deficient in the caudal midline, which then surround each ureteral orifice (which are marked in yellow).
Rotating the image 90° allows one to view a lateral image (Fig. 3) with the left side of the image representing the lumen of the bladder (blue) and the area to the right of the specimen representing the lumen of the vagina (aquamarine). The approximate thickness of the sample at the interureteric ridge is 1.8 cm with the detrusor muscle accounting for about 0.7 cm and the outer layer for about 1 cm. It is clear in this view that the nerves are most dense at the bladder neck and at the interureteric ridge and it appears that they cross between the adventitia and muscle layers at these two locations. As the nerves course between the bladder neck and interureteric ridge, they do so within the adventitial layer, close to the border with the detrusor at a depth from the luminal surface of 0.7–1.7 cm. The uniformity of detrusor thickness between the bladder neck and interureteric ridge means that this depth stays constant. At the superior border of the specimen, the density of nerves becomes sparser and no branches that penetrate the detrusor are large enough to be visible.

3D Map of Bladder Innervation in the Human Male

An AP image from the 3D map of bladder innervation in the male human bladder is depicted in Figure 4. Consistent with our findings in the female specimens, the areas of highest nerve populations appear at 3 o’clock and 9 o’clock at the bladder neck and at the interureteric ridge. In the lateral view created by rotating our map 90° clockwise around the z-axis (Fig. 5), the nerves also appear to travel predominantly within the adventitial layer close to the border with the detrusor and they cross between the two layers at the level of the bladder neck and interureteric ridge. While the appearance of nerve bands travelling between the bladder neck and ureteral orifices is apparent as it was in the female specimens, those in the male model are located somewhat more medially as they travel in a caudal–cephalad direction through the trigone. At the interureteric ridge, the depth of the nerve bands is approximately 0.5 cm. However, due to the increasing detrusor thickness as one descends caudally down to the bladder neck, this depth from the luminal surface increases to approximately 1 cm. Other than these observations, there does not appear to be a significant amount of gender-specific difference in the arrangement of the neural network in the human bladder.

DISCUSSION

The completed 3D maps demonstrated herein confirm the trigone as the bladder region that is most densely innervated. Moreover, several important new observations are apparent. Myelinated fibers are evident throughout the detrusor layer of the trigone. It is clear that in both the male and female, penetration of the nerves into the detrusor occurs predominantly at the bladder neck and at the interureteric ridge. However, as nerves travel in parallel to the luminal surface, they do so within the adventitia, or anterior vaginal wall in the
female. In a female this depth relative to the luminal surface is relatively constant, approximately 0.7–1.7 cm below the luminal surface. In the male, the depth is variable, likely owing to detrusor hypertrophy. Near the interureteric ridge, the nerves travel closer to the luminal surface, approximately 0.5 cm, while caudally near the bladder neck where the detrusor is thicker; the minimal depth is approximately 1 cm. An understanding of this anatomy is likely critical for optimizing denervation and reconstructive procedures on the lower urinary tract.

Both detrusor overactivity and underactivity are thought to be related to neural dysfunction with a particular emphasis on the afferent system. The ability to target these nerves without collateral effects elsewhere in the lower urinary tract would have enormous clinical ramifications. Chemodenervation using intravesical injections of botulinum toxin is useful for the treatment of bladder overactivity without the systemic adverse effects associated with pharmacotherapy. However, the current FDA-approved injection template is non-specific, consisting of 20–30 sites located along the posterior and lateral bladder walls beyond the trigone, and was developed prior to understanding the importance of the afferent system. A more directed injection template based on the data presented herein may allow fewer injections at a lower dose while maintaining the favorable effects of this therapy. Currently, the botulinum toxin A injection template is trigone sparing. Since botulinum toxin acts at the sensory nerve endings as well as the synaptic nerve junctions, our data suggest that the trigone is potentially the optimal region where therapy should be directed. A randomized controlled trial comparing bladder base only to bladder base and trigonal injections demonstrated that the latter produced significant improvement in post-operative symptom scores. The theoretical risk of iatrogenic vesicoureteral reflux was not realized, an observation noted by another series as well. Of course, any optimized injection template, perhaps developed from the data herein, will require validation by clinical trials. Emerging therapies for detrusor underactivity, including bioengineering approaches and gene transfection, may also benefit from this work if directed selectively at the sites of the high nerve density.

There are certain limitations to this anatomical study. The S-100 antibody used in this study reacts to the myelin coating, specifically to S100B and more weakly to S100A1 and S100A6, and so one must consider that only certain populations of nerves are shown in the generated maps. With regards to the afferent, or sensory nerves, the A-δ fibers are myelinated and therefore, figure prominently in our map. These nerves respond to passive distension and active contraction of the bladder and are the sensory component during a normal micturition cycle. They travel from the bladder and urethra to the dorsal root ganglia in the thoracolumbar region of the spine predominantly via the pelvic nerve. The other large population of afferents, the C-fibers, is unmyelinated and is therefore, not included in our model. These fibers respond to noxious stimuli and create a perception of pain. They are not typically active in the normal micturition cycle but may become predominant in pathological conditions such as interstitial cystitis and neurogenic bladder. Future refinements of these models may include pan-neuronal antibody stains to include this fiber type. Motor neurons to the bladder include: (i) the parasympathetic nerves, which are responsible for the normal voluntary detrusor contraction and subsequent emptying of the bladder; and (ii) the sympathetic nerves, which augment bladder outlet resistance during the filling/storage phase of the micturition cycle. In both types of autonomic motor neurons, the preganglionic fibers are myelinated, while the postganglionic fibers are unmyelinated and would therefore not be visualized. Preganglionic (motor) parasympathetic fibers synapse within the human bladder wall and therefore are well seen in this model. Since sympathetic neurons synapse prior to entry into the bladder wall, they are not...
visualized. Therefore, it is important to recognize that these maps only represent the afferent A-6 fibers in their entirety and the efferent preganglionic parasympathetic fibers that synapse within the bladder wall.

As a pilot study with only one specimen from each gender, our results cannot account for the role that age, ethnicity or body size and BMI may play in the variability of bladder innervation. Future studies with additional specimens will be required to answer these and other important concerns.

CONCLUSIONS

Our 3D map of human bladder innervation demonstrates that the myelinated sensory neurons and preganglionic parasympathetic fibers traverse the adventitia and penetrate the detrusor predominantly near the bladder neck at the 3 o'clock and 9 o’clock positions as well as around the ureteral orifices and interureteric ridge. This information confirms several clinical observations and provides useful knowledge for the development of interventional strategies.

REFERENCES