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ORIGINAL ARTICLE

A cadaveric histological investigation of the prostate with three-dimensional reconstruction for better results in continence and erectile function after radical prostatectomy

MB Özdemir[1], SY Eskicorapci[2], DE Baydar[3], M Cumhur[4], S Önderoğlu[4] and H Özen[5]

[1]*Department of Anatomy, Faculty of Medicine, Pamukkale University, Denizli, Turkey;* [2]*Department of Urology, Faculty of Medicine, Pamukkale University, Denizli, Turkey;* [3]*Department of Pathology, Faculty of Medicine, Hacettepe University, Ankara, Turkey;* [4]*Department of Anatomy, Faculty of Medicine, Hacettepe University, Ankara, Turkey and* [5]*Department of Urology, Faculty of Medicine, Hacettepe University, Ankara, Turkey*

In this study our aim is to increase the understanding of the prostate and related organs anatomy for better continence and erectile function results after urological surgery. Prostate and related organs were dissected from seven cadavers. After dissection, 165 serial sections with 300 μm thickness were derived at a 100 μm interval. The histological images were examined and imported to the computer. Three-dimensional (3D) remodeling had been performed. The findings were evaluated into three categories: macroscopic, microscopic and 3D reconstruction. Striated muscle fibers had been detected at the anterior fibromuscular stroma in histological sections. In 3D remodeling, urethra seemed to be a complete functional unit, beginning from the trigone up to the membranous urethra. The neurovascular bundles run under the pelvic fascia on both sides and go through to the bladder neck at 5 and 7 o'clock. Computer remodeling demonstrated that neurovascular structures had a close association with the bladder neck and the seminal vesicle. Computer program made it possible to rotate all 3D-reconstructed figures by 360° and examine them from all possible angles. All reconstructed structures can be examined together at the same time or one by one. Surgeons must pay special attention to the continence area described as a single unit, beginning from trigone to the membranous urethra, during the surgery. Meticulous dissection of the neurovascular bundles, especially close to the seminal vesicles and bladder neck, during the radical prostatectomy is necessary. These reconstructions can be used for the educational purpose of medical students as well as the urology surgeons.
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Introduction

A thorough understanding of the anatomy of the prostate and the surrounding structures is crucial for educational purposes and for the surgeons performing urological pelvic surgery. Unfortunately, prostate gland anatomy is complex. Understanding and illustrating the whole picture of the prostate and the exact relationships between the adjacent structures is challenging.[1] Moreover, urethral complex and the neurovascular structures must be defined clearly in the surgeon's mind before operation, especially for better postoperative erection and continence of the patient.[2] It will be very difficult to reach the goals of prostatectomy without an understanding of the complex anatomy of the prostate and related organs.

Correspondence: Dr SY Eskicorapci, Department of Urology, Faculty of Medicine, Pamukkale University, Denizli 20070, Turkey.
E-mail: drsye@yahoo.com
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In many textbooks, the anatomic illustrations of the prostate and related organs are either incapable or incorrect. There are many errors in illustrations and these continue with the new copies.[2] Therefore, there is a need for better illustrations of the prostate, given that prostate cancer is one of the most common cancers of men. Accordingly, radical prostatectomy is frequently performed in our country parallel to the rest of the world.[3,4]

Anatomical methods have progressively improved with new dissection instruments, stains, microscopes, cameras and photography and digital imaging systems. High-speed computers with three-dimensional (3D) graphic capabilities allow sophisticated reconstruction and manipulation of complex objects and these methods are increasingly used for medical purposes.[5]

In this study, all anatomical methods, including macroscopic, microscopic techniques and computational anatomy (3D reconstruction), were used simultaneously to define the prostate and related organs. Our aim was to increase the understanding of the anatomy of the prostate and related organs anatomy for better continence and erectile function results after urological surgery.

Materials and methods

The prostate and related organs from the cadavers used for routine dissection in education in Anatomy Department of Hacettepe University were studied. There were seven adult cadavers fixed by formaldehyde with no known diseases or abnormalities. Ages of the cadavers ranged between 45 and 65.

Dissection from cadaver
Dissection started by entering the pelvic cavity from the superior pelvic aperture. All the structures were examined by inspection and manual palpation. Once dissection was completed, the prostate and related organs were free from rectum, ureter, urinary bladder (except trigone of bladder), penis, muscles, fascias and ligaments. Neurovascular structures, trigone of bladder, ureter (last part), ductus deferens (last part), seminal gland, excretory duct, prostate, preprostatic, prostatic and membranous part of urethra and fascias that covers all the above were preserved in our specimens. Subsequently, all the specimens were placed in 10% formalin solution.

Preparation of microscopic serial sections
For 3D reconstruction, right and left sides of the specimen were marked with black- and green-colored ink, respectively. Colored inks were fixed with Bovine's solution. The specimen was cut by a section knife into 14 transverse pieces, named A to M.

After dissection, 165 serial sections with 300 μm thickness and 100 μm interval was made automatically by the aid of a Shandon 230 V EUR microtome. Sections were stained with hematoxylin and eosin by Leica autostainer for subsequent examination in light microscope.

Light microscopic examination and 3D reconstruction
All the sections were individually studied in light microscope. The preliminary examination of all the sections had been carried out to recognize the anatomical structures before 3D remodeling of the prostate. Each individual structure was marked with different colors for remodeling such as: veins, membranous urethra and trigone marked in blue; seminal vesicle and prostate in green; nerves and ejaculatory duct in yellow; arteries, external urethral sphincter and vas deferens in red; and prostatic fascia marked in white.

All borders, critical structures like muscle fibers and neurovascular bundle were marked for constructing wire frames. All wire frames had been reconstructed to form meshes. Meshes had been rendered to perform 3D reconstruction. Then, serial section's images were imported to the computer.

Surf Driver, a commercially distributed PC-based program for reconstructing 3D coordinate models from serial sections, was used for 3D remodeling.

Results

Macroscopic findings
Prostate and related organs dissected from cadaver were inspected and the crucial findings were noted. Rectoprostatic fascia, ductus deferens, seminal vesicle and bladder was shown in Figure 1a and b on dissected material. Muscle fibers of the external urethral sphincter appeared thicker at the anterior side than the posterior. The bladder seemed to be sitting on the anterior part of the prostate.

Microscopic findings
Anterior fibromuscular stroma, prostatic sinus, prostatic utricle, ejaculatory duct, capsule of prostate and prostatic urethra were defined in histological sections (Figure 2a–c). The neurovascular bundles run under the pelvic fascia on both sides, lateral and anterior to the rectum and go through to the bladder neck at 5 and 7 o'clock (Figure 2b). Striated muscle fibers had been detected at the anterior fibromuscular stroma in histological sections (Figure 2c).

3D reconstruction findings
By the aid of the computer program, the whole prostate and related structures were reconstructed. Subsequently, special structures like striated muscle fibers at anterior fibromuscular stroma and urethra were reconstructed (Figure 3a–d). We also reconstructed ureter, bladder, trigone of bladder, ductus deferens, prostate and seminal gland, ejaculatory duct, external urethral sphincter and neurovascular structures.

The distribution of the striated muscles observed at the anterior fibromuscular stroma is shown in 3D by white

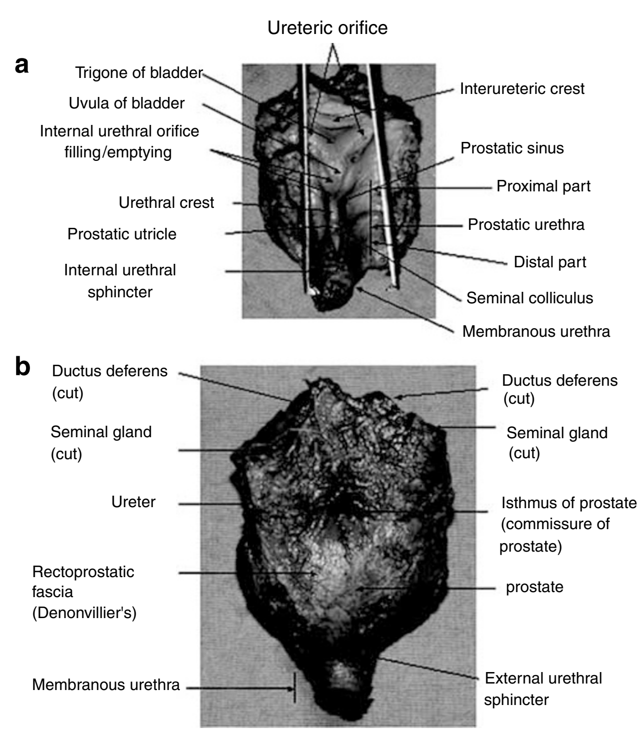

Figure 1 Macroscopic images. (**a**) Anterior view of the prostate and related organs. (**b**) Posterior view of the prostate and related organs.

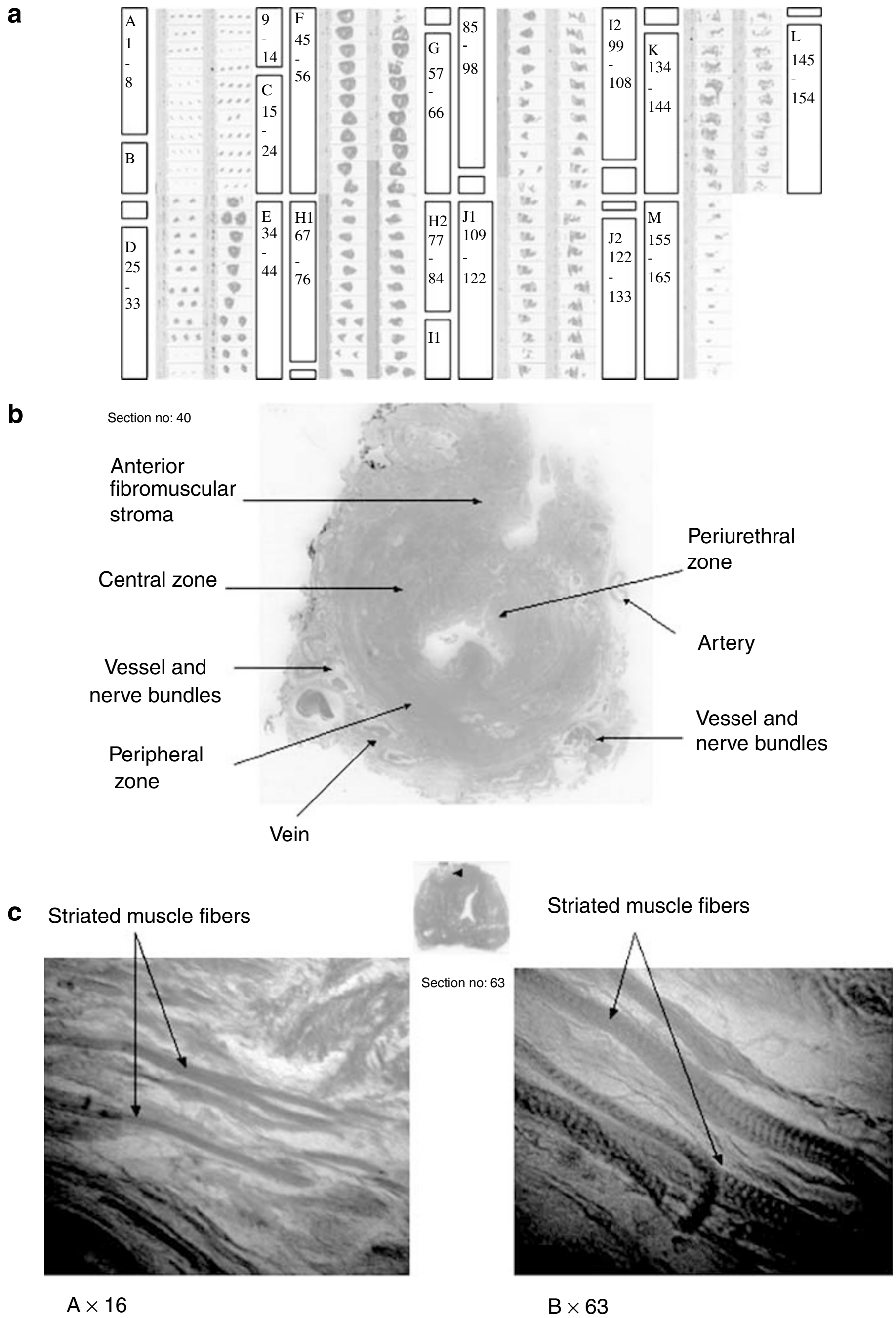

Figure 2 Microscopic images. (**a**) The level of the sections. (**b**) Localization of the neurovascular structures close to the bladder neck. (**c**) A – Striated muscle fibers (light microscope ×16). B – Striated muscle fibers (light microscope ×63).

markers (Figure 3b). In 3D remodeling, urethra seemed to be a complete functional unit, beginning from the trigone up to the membranous urethra (external urethral sphincter) (Figure 3b). This complete functional unit was circumferential without any defects from the trigone to the membranous urethra.

Neurovascular bundles were examined in histological sections and reconstructed in 3D remodeling in the computer. Computer remodeling demonstrated that neurovascular structures had a close association with bladder neck and seminal vesicle (Figure 3a, c and d).

3D remodeling confirmed the macroscopic finding of bladder sitting on the anterior part of the prostate (Figure 3a–d). All reconstructed structures can be examined together at the same time or one by one (Figure 3c). Computer program made it possible to rotate all 3D reconstructed figures by 360° and examine them from all possible angles (Figure 3d). Furthermore, all reconstructions can be magnified.

Discussion

Computer-based 3D remodeling studies began to gain popularity parallel to the developments in computer

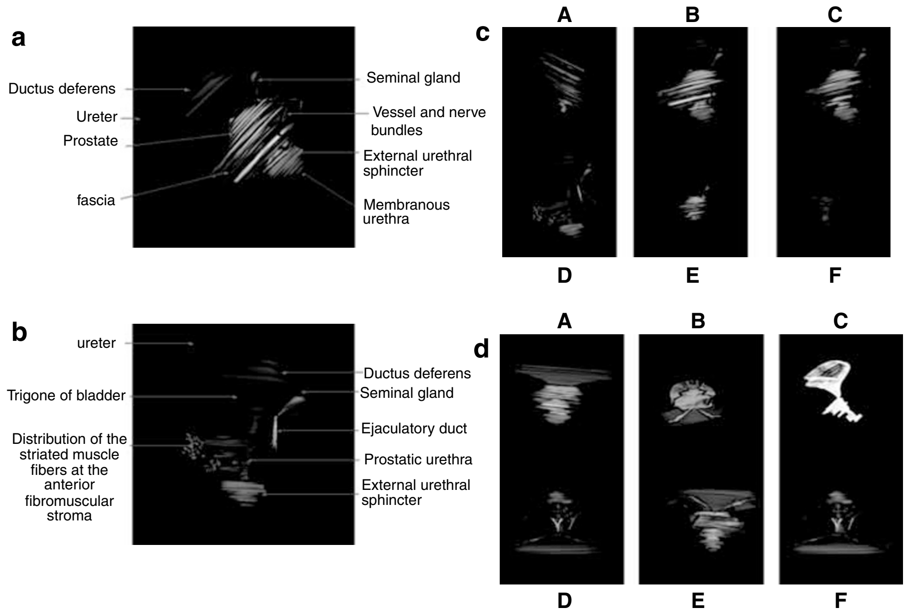

Figure 3 3D images. (**a**) 3D remodeling of the whole specimen (ureter, bladder, trigone of bladder, ductus deferens, prostate and seminal gland, ejaculatory duct, external urethral sphincter, neurovascular structures). (**b**) More detailed view of inner structures of the specimen: the distribution of the striated muscles in anterior fibromuscular stroma by white markers. (**c**) (A–F) Reconstructed structures evaluated separately. (**d**) (A–F) Reconstructed structures evaluated by rotation in computer-based program.

technology from when the prostate was shown to be of an ejaculatory duct-centric and not of a urethrocentric origin.[6] These studies might clear the doubtful points about the exact anatomy of the prostate from both surgical and educational point of view. Our aim was to increase the understanding of the complex anatomy of the prostate and related organs for better urological surgery and better education of the clinicians and persons dealing with basic sciences.

Early detection of prostate cancer had been increased mostly because of the widespread use of prostate-specific antigen.[7] Accordingly, men are generally diagnosed with prostate cancer at younger ages when they are still sexually active. Besides, most of the patients decide to have radical prostatectomy operations for the treatment of prostate cancer. Many studies showed the influence of operation techniques on especially surgical margins, incontinence and erectile dysfunction.[8,9] For a surgeon, a better understanding of the prostate gland anatomy is essential for better results. A thorough understanding of prostatic and periprostatic anatomy is crucial for the successful performance in open, laparoscopic and robotic urological pelvic surgery.[8,10,11] Urinary control and erectile function have a great influence in developing the technique of radical prostatectomy operations. Images and illustrations in our study might contribute to this purpose by making doubtful points clear in the surgeon's mind.

In the microscopic serial sections and 3D reconstructions of our series, the continence area from the trigone of the bladder to the membranous urethra (external urethral sphincter) was described as a single unit in continuity without any interruption. Recently, Yucel and Baskin[12] had similarly described the male sphincter complex from bladder neck up to membranous urethra and proximal bulbar urethra. Although our study confirmed their results, one point was different. In our study, urethral complex was continuous, whereas Yucel and Baskin[12] had shown varying degrees of separation on the dorsal aspect. This difference may be because the above-mentioned study based on fetuses in contrast to our study, which is based on adult cadavers. Furthermore, striated muscle fibers had been found not only at the urethral sphincter but also in the anterior fibromuscular stroma in our study. Their distribution was also demonstrated in 3D reconstruction. To our knowledge, the distribution of striated muscle fibers in anterior fibromuscular stroma had been shown for the first time in histological sections and 3D reconstructions. This finding also supports the above-mentioned idea of a single continence unit.

There are controversies regarding the morphological and functional role of urethral musculature. Some earlier studies stated two separate compartments, the striated and smooth muscles must be injured to cause incontinence.[13–15] However, a recent study showed that the inner smooth muscle and external sphincter muscle are developmentally inseparable.[12] Our results confirm the latter finding in two aspects. Firstly, striated muscle fibers had been found not only at the urethral sphincter but also in the anterior fibromuscular stroma. Secondly, 3D reconstructions demonstrated the continence area from the trigone of bladder to membranous urethra (external urethral sphincter) as a single unit in continuity without any interruption.

Neurovascular bundles were examined in histological sections and reconstructed in 3D remodeling in a computer. Computer remodeling demonstrated that neurovascular structures had a close association with bladder neck and seminal vesicle. Many studies in the literature questioned the exact course and structure of neurovascular bundles. Steiner[16] had found that two nerves follow separate courses. Recently, similar to the findings in our series, Karam *et al.*[17] found that the neurovascular bundle runs under the pelvic fascia on both sides lateral and anterior to the rectum and go through to the bladder neck at 5 and 7 o'clock. They also demonstrated the intimate relationship of nerve fibers with urethra and bladder neck. On the other hand, other histological studies found variations in the course of neurovascular structures.[18] Some studies indicated that nerve sparing techniques can obtain a better postoperative continence.[19] Although there are some controversies in the course and the structures of neurovascular bundles, the posterolateral course above the rectum and close relations between the seminal vesicle, urethra and bladder neck are also well established in our series. Our 3D reconstructions are unique for representing the natural course and close relations of the neurovascular structures. Our recommendation will be a meticulous dissection, especially near the seminal vesicles and bladder neck during the radical prostatectomy. More detailed studies are needed in the future for further recommendations.

As seen in macroscopic findings and 3D reconstructions, the bladder does not 'sit' directly on top of the prostate as illustrated in many classic textbook drawings.[1] Rather, the bladder is positioned anterior to the prostate. This finding has been confirmed by recent studies.[1,12]

In our opinion, adult cadaver reconstruction studies are better than studies using fetuses or magnetic resonance, as cadavers represent the adults with excellent preservation of anatomical structures with little distortion. Besides, our study is unique, as it is a complete work of macroscopic, microscopic histological examination and 3D reconstructions. In addition, it is possible to have a further insight into the relationship of pelvic structures through real-time rotation of these 3D images in all angles.

Our study investigated the histology and 3D reconstructed models of the prostate for better results in continence and erectile function. More detailed analysis has to be carried out in the future. Prostate and pelvis fascias are still to be investigated, as there is no consensus on that topic. Furthermore, more detailed analysis of neurovascular bundles must be carried out with the aid of developing computer technology.

In many textbooks, the anatomic illustrations of the prostate and related organs are either incapable or incorrect. Errors in illustrations are responsible for copying without substantiating validity. The most reasoned use of computer-based instructional resources remains in judiciously integrating them with a variety of other anatomical teaching methods. These images are basic for an advanced search and may be easily shared by the aid of computers or the Internet. It is true that these improve the understanding of the prostate and related organs. These reconstructions may totally change the education of medical students as well as the urology surgeons.

Conclusions

In the microscopic serial sections and 3D reconstructions of our series, the continence area beginning from trigone to the membranous urethra was described as a single unit in continuity. Furthermore, striated muscle fibers had been found not only at the urethral sphincter but also in the anterior fibromuscular stroma. This finding also supports the above-mentioned idea of a single continence unit.

Computer remodeling demonstrated that neurovascular structures had a close association with the bladder neck and the seminal vesicle; thus, there is a need of meticulous dissection, especially near the seminal vesicles and the bladder neck, during radical prostatectomy.

These reconstructions may totally change the education of medical students as well as the urology surgeons.

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