79

Wallner C[1], Wallace C[2], Maas C[3], Lange M[4], Lahaye M[5], Moayeri N[6], Groen G[6], Dabhoiwala N[7], Bleys R[8], Lamers W[1], DeRuiter M[9]

1. *Department of Anatomy & Embryology, Academic Medical Center, Amsterdam, The Netherlands,* ***2.*** *Department of Anatomy, Leiden University Medical Centre, Leiden, the Netherlands,* ***3.*** *Department of Gynaecology, Leiden University Medical Centre, Leiden, the Netherlands,* ***4.*** *Department of Surgery, Leiden University Medical Centre, Leiden, the Netherlands,* ***5.*** *Department of Radiology, University Hospital Maastricht, Maastricht, the Netherlands.,* ***6.*** *Department of Anesthesiology and Pain Treatment, University Medical Center Utrecht, Utrecht, the Netherlands,* ***7.*** *Department of Urology, Academic Medical Center, Amsterdam, The Netherlands,* ***8.*** *Department of Pharmacology & Anatomy, University Medical Center Utrecht, Utrecht, the Netherlands,* ***9.*** *Department of Anatomy & Embryology, Leiden University Medical Center, Leiden, The Netherlands*

A HIGH RESOLUTION 3D STUDY OF THE FEMALE PELVIS REVEALS IMPORTANT ANATOMICAL AND PATHOLOGICAL DETAILS OF THE PELVIC FLOOR

Aims of study

Human sectional anatomy initiatives such as the visible human projects of the USA, China and Korea have provided extraordinary insights into human anatomy. The use of serial sections has important advantages over conventional cadaver dissection. Dimensions and surfaces can be easily measured and topographic relations remain undisturbed. In this study we combined several visualization techniques on the basis of serial sections to obtain a comprehensive anatomical database of the female pelvis. Important anatomical and pathological observations on the pelvic floor could be made from the investigated pelvis.

Study design, materials and methods

Medical history of patient - Patient was a multiparous female, deceased at age 69 whose body was available to us for medical research. Medical history was gleaned post-mortem through contact with the family physician with permission of the patient's husband. She was diagnosed with Multiple Sclerosis (MS) 20 years prior to her death. The patient had suffered from stress urinary incontinence but not fecal incontinence.

Production of serial sections - After fixation and MRI examination the pelvis was cut into sections using a cryomicrotome. The pelvis was first frozen and subsequently cut into a 45x15x16cm block (lateral x ventro-dorsal x cranio-caudal dimension). This block contained the anatomical small pelvis. The pelvic block was then embedded and frozen in carboxymethylcellulose gels at -25˚C to then be cut into serial transverse sections of 25 µm. By placing cellophane tape onto the cutting surface of this pelvic block histological sections were obtained for (immuno)histochemical staining.

Image capturing - Digital images were taken of every third section (every 75 µm) of the frozen cut surface. A complete series of 2051 images was obtained with a resolution of 3040 x 1961 pixels per image (6 Megapixels). The size of a single pixel was 82 µm. Image was saved in tiff format (17.5 MB/image), resulting in a 35.9 GB data-set.

Computational production of images in the sagittal and coronal plane - Digital images in the coronal and sagittal plane were produced from the transverse digital image data set using the 'Enhanced Multiplanar Reformatting Along Curves'-technique (E-MAC group, Department of Information and Computing Sciences, University of Utrecht, Netherlands). This way a series of 1744 coronal images (2648 x 2048 pixels, 5.4 megapixels) and a series of 2648 sagittal images (1744 x 2048 pixels, 3.6 megapixels) were obtained, resulting in an additional data-set 55.4 GB.

(Immuno)histochemistry - At levels of interest every 0.5 mm, tape-collected tissue sections were stained (immuno)histochemically to demonstrate the different tissue compositions. Sections were stained immunohistochemically with an antibody directed against striated muscle myosin heavy chain (monoclonal mouse antibody, Upstate, clone A4.1025; 1:2000) to visualize striated muscle tissue (1,2). Sections were also stained histochemically for collagen using picro-sirius red staining and with Mallory-Cason trichrome staining for overview.

3D-reconstruction from serial sections - Digital images from the transverse serial sections were used to prepare 3D reconstructions with the Amira software package (TGS). Using 'Adobe Acrobat 3D' we were able to convert our 3D reconstruction into a 3D pdf file. The 3D pdf contains a fully interactive 3D reconstruction that can be freely rotated and zoomed in and out. Also all structures can be independently turned on and off for evaluation and study (1,2).

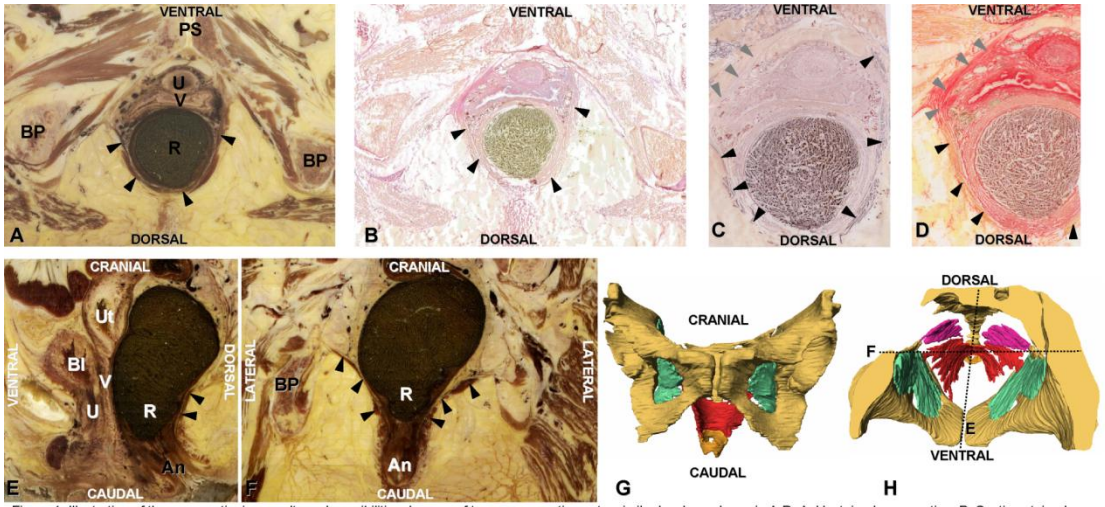

Figure 1. Illustration of the cryo-sectioning results and possibilities. Images of transverse sections at a similar level are shown in A-D. A. Unstained cryo-section. B. Section stained histochemically with Mallory-Cason trichrome stain. C. Immunohistochimically stained section stained for striated muscle (blue color). Note blue staining of the striated levator ani muscle (black arrowheads) and the lack of LAM tissue on the left side of the urethra and vagina (grey arrowheads). D. Section stained histochemically for Sirius Red stain for connective tissue. Note the connective tissue fascia (grey arrowheads) where the levator ani muscle (LAM) in lacking. The LAM is illustrated by the black arrowheads in A-F. The serial transverse images (example in A) are used to produce a series of digital sagittal (E) and frontal (F) images of the same pelvis in identical resolution as the transverse images. A 3D reconstruction was produced from the transverse serial sections (G, frontal view; H, superior view). The levator ani muscle is demonstrated in red. Note that the position of the computed sagittal section (E) and frontal section (F) is illustrated by the dotted lines in H. An, anus; Bl, bladder; BP, bony pelvis; PS; pubic symphysis; R, rectum; U, urethra; Ut, uterus; V, vagina.

Results

Careful investigation of the MRI, serial histological and (immuno)histochemically stained sections revealed a gross unilateral levator ani muscle (LAM) defect (Fig. 1A-D, H). The muscular attachment to the pubic bone on this side was completely lacking. Interestingly, the fascial connection of the muscle to the pubic bone is still present, as illustrated by sections stained for collagen (Fig. 1D). The vagina was asymmetric as seen in the transverse sections (Fig. 1A). The unilateral absence of the levator ani muscular tissue connecting to the pubic bone is also visible in the 3D recosntruction (Fig. 1G/H). Patient did not suffer from pelvic organ prolapse and this can also be confirmed from the computationally obtained mid-sagittal image (Fig 1E). Fig. 1F illustrates the LAM (black arrowheads) in a coronal image at the level of the ischial spine. Note that at this level the levator ani is present on both sides.

Interpretation of results

Our data-set of a female pelvis contains more elaborate information on pelvic anatomy than previously published full body data sets, such as the Visible Human Projects of the US, China and Korea. Such a detailed amount of information can only be produced through a combination of several techniques like MRI, high resolution digital transverse serial images, computationally obtained high resolution digital coronal and sagittal serial images, as well as (immuno)histochemically stained sections and 3-D reconstruction. Additionally, we used a camera with a higher resolution (6 megapixels) than previous visible human data-sets. This resulted in a very detailed 3D data set with a voxel size of 82 x 82 x 75 µm. Also, we were able to verify the sections used for our 3D reconstruction with (immuno)-histochemically stained sections. This allowed us to clearly identify and reconstruct all the structures in the pelvis, such as the muscular components of the LAM and its fascias. This was impossible in previous reconstructions which were obtained using the slices from the Visible Human Project. LAM damage as seen in this study has previously been visualised using MRI and CT and can occur during vaginal delivery. This LAM damage is known to cause incontinence and pelvic organ prolapse. The cause of LAM damage in this investigated pelvis is most likely due to vaginal deliveries in the past. Due to the patient's history of MS, one cannot eliminate completely a neurological contribution to some atrophy of the LAM.

Concluding message

A combination of techniques used by us in this project give a unique insight into the composite elements of the human pelvis and the various relationships that exist between the pelvic organs and their supports which can not be matched by data sets such as produced by the Visible Human Project. To our knowledge this is the first anatomical description and histological confirmation of LAM damage in an individual with a history of stress incontinence.

References

1. Wallner C, Dabhoiwala NF, DeRuiter MC, Lamers WH (2009). The anatomical components of urinary continence. Eur Urol 55(4), 932-44
2. Wallner C, van Wissen J, Maas CP, Dabhoiwala NF, DeRuiter MC, Lamers WH (2008). The contribution of the Levator Ani Nerve and the Pudendal Nerve to the innervation of the Levator Ani Muscles; a study in human fetuses. Eur Urol 54(5):1136-42

Specify source of funding or grant	**This study is supported by a grant from the John L. Emmett Foundation for Urology, The Netherlands.**
Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**No**
This study did not require eithics committee approval because	**only cadaver material was used.**
Was the Declaration of Helsinki followed?	**No**
This study did not follow the Declaration of Helsinki in the sense that	**only cadaver material was used.**
Was informed consent obtained from the patients?	**No**