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Kraima, AC, West, NP orcid.org/0000-0002-0346-6709, Treanor, D et al. (6 more authors) (2016) The anatomy of the perineal body in relation to abdominoperineal excision for low rectal cancer. Colorectal Disease, 18 (7). pp. 688-695. ISSN 1462-8910

https://doi.org/10.1111/codi.13138

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Received Date : 10-Mar-2015

Revised Date : 17-Jul-2015

Accepted Date : 24-Jul-2015

Article type : Original Article

169-2015.R2 Original Article

The anatomy of the perineal body in relation to abdominoperineal excision for

low rectal cancer

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Conflict of interest: none

Word count body of text: 2.992 (including references in square brackets).

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/codi.13138

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Abstract

Aim: Dissection of the perineal body (PB) during abdominoperineal excision (APE) for low rectal cancer is often difficult due to lack of a natural plane of dissection. Understanding of the PB and its relation to the anorectum is essential to permit safe dissection during the perineal phase of the operation, to avoid damage to the anorectum and urogenital organs. This study describes the anatomy and histology of the PB relevant to APE.

Method: Six human adult cadaver pelvic exenteration specimens (three males, three females) from the Leeds GIFT Research Tissue Programme were studied. Paraffin-embedded megablocks were produced and serially sectioned at 50 and 250 µm intervals. Sections were stained by immunohistochemistry to show collagen, elastin and smooth muscle.

Results: The PB was cylindrically-shaped in males and wedge-shaped in females. Although centrally located between the anal and urogenital triangles, it was nearly completely formed by muscle fibres derived from the rectal muscularis propria. Thick bundles of smooth muscle mostly arising from the longitudinal muscle, inserted into the PB and levator ani muscle (LAM). The recto-

urethralis muscle originated from the PB and separated the anterolateral PB from the urogenital organs.

Conclusion: Smooth muscle fibres derived from the rectal muscularis propria extend into the PB and LAM and appear to fix the anorectum. Dissection of the PB during APE is safe only when the smooth muscle fibres that extend into the PB are divided.

What does this study add to the literature?

This is the first study reporting the anatomy and histology of the perineal body relevant to a surgical oncological approach. The anterior rectal muscularis propria contributes most to the formation of the PB and forms a strong fixation of the anorectum to the perineal body and pelvic floor.

Introduction

Total mesorectal excision involves *en-bloc* removal of the rectum and surrounding mesorectum [1]. The object is to achieve a tumour-free circumferential resection margin (CRM) to reduce the risk of local recurrence [2]. Patients with low rectal tumours requiring an abdominoperineal excision (APE) may have a poor prognosis [3]. The operation is associated with higher rates of CRM involvement, tumour perforation and incomplete surgical removal compared with anterior resection for more superiorly located rectal tumours [4]. One of the most difficult steps in APE is dissection at the level of the perineal body (PB).

The PB is located in the midline of the perineum at the junction between the anal and urogenital triangles [5]. There is no anatomical plane through the PB along which would permit surgical cleavage. The PB was first named in 1889 [6] and has been mainly studied in relation to obstetric injuries [7-11]. There are only a few studies of the histology of the PB and these are conflicting as there is no consensus on its relationship to the adjacent muscles. There are no descriptions relating the anatomy to a surgical oncological approach.

Due to its central position, incorrect dissection of the PB could lead to anorectal and urogenital injury risking the functional and oncological outcome of patients having an APE. Thus it is essential to understand the anatomy of the PB and its intricate relationship with the anorectum. In this study, we aimed to define the anatomy and histology of the male and female perineal body and in the light of this to describe the possible implications for the anaterior dissection during APE for low rectal cancer.

Method

Adult cadaveric specimens

Through the University of Leeds GIFT Research Tissue Programme (www.gift.leeds.ac.uk), six human adult cadaveric specimens were obtained from donors who consented before death. Ethical approval was granted by the Northern and Yorkshire Regional Ethics Committee, Jarrow, UK (unique reference number 11/H0903/6). The donor bodies belonged to three males (68, 89 and 99 years) and three females (63, 64 and 74 years). All female bodies had a history of childbirth. None

of the donor bodies had had any known pelvic pathology. The specimens were retrieved during tissue donation autopsies performed in the prone jack-knife position according to the technique described by Hölm et al [12] at St. James's University Hospital in Leeds. Essenitally they were complete pelvic exenterations, which included the anal canal and rectum up to the rectosigmoid junction, the mesorectum with an intact mesorectal fascia, levator ani muscle, obturator internus muscle, posterior bladder wall, vagina or prostate and penile bulb, the anal sphincter complex and the perineal body. All specimens were fixed in formalin [8%] solution for seven days before transverse sectioning at a thickness of one centimetre. The slices were photographed and dissected to fit in Super Mega Cassettes measuring 74.8 x 52.5 x 16.5mm (CellPath; Powys; UK). The tissues underwent an extended tissue processing cycle in a Leica ASP200 tissue processor as follows: 1 hour (h) in 70% ethanol, 2 h in 80% ethanol, 2 h in 90% ethanol, 3 h in 95 % ethanol, 12 h in 100% ethanol (repeated three times), 12 h in xylene, 24 h in xylene (repeated twice), 24 h in paraffin. All tissues were embedded in paraffin mega blocks.

Immunohistochemical staining

The mega blocks were transversely sectioned in slices 5 μ m thick. In one male and one female specimen, every tenth section was placed on to a glass slide and stained with haematoxylin and eosin (H&E), creating a series of sections at an interval of 50 μ m. Additional sections were collected from each mega block and stained using Masson's trichrome (MT) and Miller's elastin (ME) [13]. In the other male and female specimens, every 48th, 49th and 50th section were collected, to create three series with a cross-sectional interval of 250 μ m, of which one series was stained with H&E and one with MT. The remaining section was reserved for additional stains with ME.

Selected sections from one female specimen were stained immunohistochemically with a mouse monoclonal antibody against α -smooth muscle actin, which was diluted to 1 in 500 (M0851, DAKO, Cambridge, UK), to reveal smooth muscle fibres. The Menarini X-Cell Plus Kit was used and all following products were derived from Menarini Diagnostics Ltd., Winnersh-Wokingham, UK. Deparaffinization and antigen retrieval was performed in a Menarini Access Revelation solution diluted 1 in 10 in deionized water at 125 C for two minutes after which sections were cooled down to 90°C. Between all steps, sections were rinsed in Intellipath automation wash buffer. Endogeneous peroxidase activity was blocked in a Menarini peroxide block for ten minutes at room temperature. In consecutive steps, sections were blocked with a Menarini block solution with casein for ten minutes, incubated in primary antibody diluted in Zymed diluent (Life Technologies Ltd., Paisley, UK) for 60 minutes, incubated in Menarini Universal Probe for 30 minutes, incubated in polymer horseradish peroxidase for 30 minutes and incubated in Menapath DAB solution for five minutes, all at room temperature. After this, sections were counterstained with Mayers' haematoxylin for one minute, differentiated in Scott's water, dehydrated in ethanol, cleared in xylene and mounted with DPX.

Image acquisition

The stained glass slides were scanned in Leeds with an Aperio XT slide scanner (Aperio, San Diego, California, USA) at 20x magnification, creating a resolution of 0.46 microns per pixel. The digital images were compressed with JPEG2000 quality 70 and viewed in Aperio ImageScope (version 10.2.2.2319).

We used the nomenclature of *Gray's Anatomy* [5] to refer to the different muscles and fasciae. As the pelvic cadaveric specimens have been detached from the bony pelvis, they did not include the deep and superficial transverse perineal muscles.

Statistical Analysis

Results

The male perineal body

The male PB appeared as a cylindrical fibromuscular tissue mass, containing an abundant amount of densely-packed collagen. Microscopically, small arterioles, venous plexuses and ramifications of the pudendal nerve were visible throughout the whole PB. The PB was closely related to the membranous portion of the posterior urethra and the anterior anorectum. The most caudal part of the PB, located just underneath the central perineal skin, was less densely-packed and contained more areas of adipose tissue. The external anal sphincter (EAS) consisted of two parts, including a caudal part that encircled the complete anal canal and a cranial part that was non-continuous anteriorly. The PB formed an integral part between the caudal portion of the external anal sphincter (EAS) and the apex of the prostate, covering the anterior part of the anorectum. The most cranial part of the PB was attached to Denonvilliers' fascia, also known as the rectogenital septum.

Table 1 summarizes the anatomy of the insertion of striated and smooth muscles into the PB. The EAS, bulbospongiosus muscle (BM), external urethral sphincter and levator ani muscle (LAM) were all striated muscles that anchored into the PB. The longitudinal muscle (LM) of the rectum and anal

canal, the internal anal sphincter (IAS) and the recto-urethralis muscle were all connected to the PB. At the level of the perineal membrane, the LM belonging to the rectal muscularis propria was strongly thickened and gave off multiple smooth muscle fibres that anchored into the PB and the levator ani muscle. At a microscopic level, the PB was nearly completely taken up by smooth muscle fibres from the LM (Figure 1).

Two major bilateral strands of smooth muscle fibres from the LM formed the recto-urethralis muscle (Figure 2). This muscle ran through the PB and was anterolaterally adherent to the LAM and posteriorly to the bulbourethral glands (Cowper's gland; Figure 1). In two specimens, the IAS was thickened at a level below the thickening of the LM and intermingled with the LM and anchored into the PB. In the other specimen, the IAS was not thickened and remained thin relative to the LM. All specimens revealed an intimate relationship between the dorsal part of the PB and the anal submucosa and the anal canal. The LAM anchored laterally into both sides of the PB along its vertical length (figure 2).

The female perineal body

The female PB was thicker and wider compared with the male. It appeared as a wedge-shaped rather than cylindrical fibromuscular tissue mass. Small arterioles, venous plexuses and ramifications of the pudendal nerve were microscopically detected throughout the whole PB. The venous plexuses were extensive along the entire posterior vaginal wall. In contrast with the male, the female PB was more densely-packed with collagen. It was located directly underneath the perineal skin and posterior to the introitus of the vagina. The EAS had a similar appearance in the

female specimens, consisting of a caudal and cranial portion, of which the latter was anteriorly noncontinuous. The PB occupied the anterior gap and formed an integral part between the cranial part of the EAS and posterior vaginal wall, covering the anterior aspect of the anorectum. The upper border of the female PB could not be precisely identified as it gradually disappeared along the posterior vagina into Denonvilliers' fascia.

The histology and topography of the muscles that anchored into the female PB were comparable to the male PB (Table 1). Also in the female specimens, the LM and IAS were strongly thickened at the level of the perineal membrane, intermingling with each other and protruding into the PB (Figure 3). The urethrovaginalis was a smooth muscle structure that originated from the PB and passed forwards on either sides of the vagina. Due to the absence of the urethra in the female specimens, we were unable to follow its complete course.

Discussion

The perineal body is one of the less understood anatomical structures in the perineum. Although it has been previously studied on several occasions, its detailed anatomy is still unclear, particularly in relation to the question of oncological dissection during abdominoperiineal excision of the rectum.. By using different immunohistological stains in whole mount sections of male and female cadaveric pelvic specimens, we could accurately study the anatomy and histology of the PB and describe the implications of the findings on the anterior dissection of APE.

The present study has demonstrated histologically that the muscularis propria of the rectum extends into the PB and forms a major component of this structure. Smooth muscle fibres extending directly

from the LM and to a lesser extent the IAS have major projections in an anterolateral direction, strongly binding the anorectum to the PB and the pelvic floor. Although the PB is located centrally between the anal and urogenital triangles, it is nearly fully composed of smooth muscle fibres extending from the rectal muscularis propria. In contrast the muscles of the urogenital triangle anchor into the anterolateral sides of the PB rather than occupying it.

The perineal phase of an APE is one of the most difficult dissections in colorectal surgery as surgeons must find their way through the PB to separate the anal canal and lower rectum from the anterior structures. Macroscopically, the PB appears as a poorly defined mass located anterior to the anal sphincter complex. Microscopically it consists of densely-packed collagen into which smooth muscle fibres from the LM and IAS anchor. This results in an intricate relationship between the PB and the anterior rectal muscularis propria, the underlying anal submucosa and the anal canal. Moreover, the present study has shown histologically that the PB is closely related anteriorly to the membranous portion of the posterior urethra and penile bulb in males and is actually fused with the posterior vaginal wall in females. Surgical dissection of plane through the PB too dorsally, risks dissecting into the rectal muscularis propria or even the anal submucosa, which may result in a non-rradical resection or an intraoperative perforation. Conversely if the plane is too ventral, the dissection risks damaging the membranous portion of the posterior urethra or penile bulb or the posterior vaginal wall., where the extensive venous plexus is at risk of damage with the possibility of bleeding.

How should the PB be optimally dissected during abdominoperineal excision of the rectum ? Heald et al. [14] advocated that it should take place posterior to the superficial transverse perineal muscle, ensuring complete removal of the anal sphincter and the anteriorly directed smooth muscle fibres of the rectal wall. The cadaveric pelvic specimens in the present study did not include the superficial transverse perineal muscles, wherefore the description of Heald et al could not be fully verified. We did show, however, that the anteriorly directed smooth muscle fibres from the rectal muscularis propria firmly attach into the PB. The PB fills the anterior gap of the cranial part of the EAS and forms an integral part between the anal and urogenital triangles.

Based on these histological observations, the perineal phase of an abdominoperineal exci9sion should first to mobilise the dorsal and lateral parts of the anal sphincter complex, as in fact has been the practice for many years. The dissection should start just anterior to the EAS and continue cranially, dividing only those smooth muscle fibres that extend from the LM to the PB. Clearly it is most important to keep enough distance from the rectal muscularis propria to avoid dissection in the LM and IAS and to stay posterior to the recto-urethralis muscle so as not to damage the urogenital organs. The dotted lines in Figures 1, 2 and 3 mark the preferred dissection planes.

Additionally, Figure 4 reflects the surgeon's view and shows the initial steps of the perineal phase of an APE with the patient in the supine position. Through full exposure of the PB the macroscopic extent of the PB can be seen and its relationship to the EAS can be determined. Gentle backward pressure on the specimen stretches the anteriorly directed smooth muscle fibres from the LM after which the PB can be dissected more safely by staying between the rectal muscularis propria and the recto-urethralis muscle. It should be kept in mind, at least in females, that previous surgery or the presence of a rectocele could increase the adherence of the anorectum to the PB. The close

adherence of the PB to the anal and urogenital triangles can be explained embryologically. In early development, the urorectal septum, from the tip of which forms the perineum, separates the cloaca into a dorsal anal canal and a ventral urogenital sinus. As a consequence, the PB is the central point into which muscles of the anal and urogenital triangles insert [15].

The microscopic anatomy of the PB has been previously described [11, 16, 17]. Oh and Kark [11] examined histologically the insertion of muscles and fasciae of the urogenital diaphragm into the PB, focussing predominantly on the male. Although the extension of the rectal wall into the PB was briefly mentioned, the important role of the LM was not acknowledged. The lower part of the PB was referred to as a region where the EAS, superficial transverse perineal muscles and BM decussate, whereas we have shown that these muscles do not actually cross, but rather anchor into the dorsolateral and anterolateral sides of the PB. Shafik et al proposed a new concept of the anatomy of the PB by referring to it as the site across which muscles uninterruptedly passed from one side to the other in an intertwining criss-cross pattern [16]. Based on cadaveric dissections, the PB was described as having a 'digastric pattern', consisting of three layers: 1) a superficial layer, formed by the EAS extending across the PB to become continuous with the BM, 2) a middle layer, composed of tendinous extensions of the superficial transverse perineal muscle that decussated in the PB and 3) a deep layer, composed of tendinous extensions of the deep transverse perineal muscle that crossed in the PB. The present study could not confirm these three layers nor the continuum between the EAS and BM nor the 'digastric pattern'. Instead the PB appeared histologically as a fibromuscular tissue mass that appeared to be a central fixation point rather than a site of the crossing of fibres. Soga et al [17] reported that the lateral extensions of the PB maintained the topographical relations with the vagina and rectum, but they did not include the male PB in their

histological analysis. Unofrtunately it was not possible in the present study to examine the lateral attachments of the PB as the cadaveric specimens were detached from the bony pelvis. Their use has further limitations as they may have suffered from topographical distortion due to fixation and loss of muscle tone.

Other researchers have examined the PB by thin-slice magnetic resonance imaging [18] or by *in vivo* surgical observations [19-21]. Wagenlehner et al [19, 20] have showed that the PB could be divided into two parts, which were connected via a central tendon, but it was not possible to examine the two parts of the PB in the present study. The functional role of the PB has to be explored *in vivo*. To reveal the detailed anatomy of the PB and its intricate relation to the surrounding structures it is crucial to perform histological assessment of the tissues. Dissection in a cadaver of an area rich in dense connective tissue could easily lead to the creation of artefacts. Radiologic imaging and *in vivo* observations might be helpful in understanding the functional role of the PB and its topographical relations, but they cannot identify sufficient anatomical detail owing to the limited resolution of the technique.

In conclusion, the perineal body is a fibromuscular mass located between the anal and urogenital triangles. Surgeons should be aware of the extent to which smooth muscle fibres derived from the rectal muscularis propria extend to the PB and LAM, creating a strong fixation of the anorectum to the perineum and pelvic floor. During dissection of the PB during APE, it is most important to divide only the smooth muscle fibres that pass to the PB, whilst keeping a safe distance from the rectal wall and the anteriorly located urogenital organs.

Acknowledgements

We would like to thank the GIFT donors who made this study possible and without whom no progress could be made in this area. Martin Waterhouse, Dave Turner, Mike Hale and Daniel Jansma are gratefully acknowledged for their help in scanning the sections and processing the images. Aidan Hindley is gratefully acknowledged for his help in retrieving and administrating the GIFT specimens. This study is funded by Technology Foundation STW (grant number 10903). NW is funded by a Pathological Society of Great Britain and Ireland Career Development fellowship, an Academy of Medical Sciences starter grant for clinical lecturers and the National Institute for Health Research. PQ is funded by Yorkshire Cancer Research and the Experimental Cancer Medicines Centre initiative. The Aperio scanners were purchased with assistance from the MRC Bioinformatics Centre (grant number MR\L01629X\1).

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Muscle	Histology	Topology	
External anal sphincter	Striated muscle	Adheres posterolaterally to the perineal body	
Bulbospongiosus muscle	Striated muscle	Adheres anterolaterally to the perineal body	
Longitudinal muscle	Smooth muscle	Strongly adheres anteriorly and anterolaterally to the perineal body and levator ani muscle	
Recto-urethralis muscle	Smooth muscle	Originates from the longitudinal muscle extending into the perineal body, adheres anterolaterally to the levator ani muscle and posteriorly to the bulbourethral glands	
Internal anal sphincter	Smooth muscle	Intermingles with the longitudinal muscle and adheres posteriorly into the perineal body	
External urethral sphincter ¹ Sphincter urethrovaginalis ²	Striated muscle	Adheres anteriorly to the perineal body	
Levator ani muscle	Striated muscle	Adheres laterally into the perineal body along its whole vertical length	

Table 1: muscles inserting into the male and female perineal body

Add 1: In the male perineal body

Add 2: In the female perineal body

Figure 1

Figure 1a shows an overview of the centre of the male perineal body (PB) in a cadaveric specimen of a 68 year old male at the level of the anorectum (A). **Figure** 1 al shows the extension of the longitudinal muscle (LM) and, to a lesser extent, the internal anal sphincter (IAS) into the PB. Dorsally, the PB is bounded by the LM and IAS. Anteriorly, the PB is bounded by the recto-urethralis muscle (RM) and Cowper's glands (CG). Note the close relationship of the PB to the corpora cavernosa (CC) and the anal submucosa (Sub). **Figure** 1a.II shows the relation of the RM to the LM and levator ani muscle (LAM). The dotted lines in windows 1a.I and 1a.II marks the line of dissection for safe division of the PB. ME: Miller's elastin. Scale bar in window (a) 6 mm; **Figure** 1a.I and 2mm **Figure** 1a.II.



Figure 2a shows an overview of the upper limit of the male perineal body (PB) in the same cadaveric specimen as in Figure 1. **Figure** 2a.I shows that the longitudinal layer (LL) of the rectal muscularis propria starts to thicken at the level of the low rectum (R) and projects anteriorly and anterolaterally into the PB. The arrow in **Figure** 2a.I illustrates the rectourethralis muscle (RM) originating from the smooth muscle fibres of the LL that extend into the PB. The external urethral sphincter (EUS) is horseshoe shaped and anchors into the anterolateral sides of the PB. Note the close relation of the upper limit of the PB to the urethra (U) and anal submucosa (Sub). The arrows in **Figure** 2a.II shows that the smooth muscle fibres from the rectal muscularis propria anchor into the levator ani muscle (LAM). The RM is bounded laterally by the LAM. The dotted line in detail **Figure** 2a.I marks the safe line of dissection to mobilise the anorectum.

CL: circular layer of the rectal muscularis propria; PR: picrosirius red. Scale bar in window 2a 6 mm; **Figure** 2a.I) 2 mm; **Figure** 2a.II 600 µm.



Figures 3a and 3b are successive sections of the perineal body (PB) in a cadaveric specimen of 74 year old female at the level of the anorectum (A). Note that the PB is wedge-shaped and less delineable compared with the male as it is fused to the posterior vaginal wall. Figure 3a.I shows the anteriorly directed smooth muscle fibres from the internal anal sphincter (IAS) anchoring into the PB. Figure 2b shows how the smooth muscle fibres from the rectal muscularis propria fix the anorectum to the PB. Window 2b.I shows that the IAS intertwines with the longitudinal muscle (LM) in the midline. Note in Figure 2a that the cranial part of the external anal sphincter is not continuous anteriorly. The dotted line in window 2a.I marks the correct dissection plane to mobilise the anorectum safely. The dissection must take place on the outer muscular layer of the rectal wall so as not to damage the posterior vaginal wall.

ME: Miller's elastin. Scale bar in Figure 3a and Figure 3b 6 mm; Figure 3a.I and 3b.I 3 mm.



Figure 4

Figures 4a, 4b and 4c show p;hotographs with line diagrams of the consecutive steps of the perineal phase of an abdominoperineal excision with the patient in the in supine position. Good exposure of the perineal body (PB) is essential to examine its macroscopic extent and to determine its relationship to the anal sphincter complex (ASC) and the mesorectum (M) as demonstrated in Figure 4b. The dotted line marks the correct plane of dissection which should lie just anterior to the external anal sphincter. Surgical dissection should start lateral to the PB to allow safe division of the fibromuscular mass, thereby avoiding damage to the rectal wall or urogenital organs (Figure 4c).

