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Whole mount microscopic sections reveal that Denonvilliers' fascia is one entity and adherent to the mesorectal fascia; implications for the anterior plane in total mesorectal excision?

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Abstract

Background: Excellent anatomical knowledge of the rectum and surrounding structures is essential for total mesorectal excision (TME). Denonviliers' fascia (DVF) has been frequently studied, though the optimal anterior plane in TME is still disputed. The relationship of the lateral edges of DVF to the autonomic nerves and mesorectal fascia is unclear. We studied whole mout microscopic sections of *en-bloc* cadaveric pelvic exenteration and describe implications for TME.

Methods: Four donated human adult cadaveric specimens (two males, two females) were obtained from the Leeds GIFT Research Tissue Programme. Paraffin-embedded mega blocks were produced and serially sectioned at 50 and 250 μm intervals. Sections were stained with haematoxylin & eosin, Masson's trichrome and Millers' elastin. Additionally, a series of eleven human fetal specimens (embryonic age of 9–20 weeks) were studied.

Results: DVF consisted of multiple fascial condensations of collagen and smooth muscle fibres and was indistinguishable from the anterior mesorectal fascia and the prostatic fascia or posterior vaginal wall. The lateral edges of DVF appeared fan-shaped and the most posterior part was continuous with the mesorectal fascia. Fasciae were not identified in fetal specimens.

Conclusion: DVF is adherent to and continuous with the mesorectal fascia. Optimal surgical dissection during TME should be carried out anterior to DVF to ensure radical removal, particularly for anterior tumours. Autonomic nerves are at risk, but can be preserved by closely following the mesorectal fascia along the anterolateral mesorectum. The lack of evident fasciae in fetal specimens suggested that these might be formed in later developmental stages.

Keywords: Total mesorectal excision; Denonvilliers' fascia; Rectal cancer; Mesorectal fascia; Autonomic nerves

Introduction

The introduction of total mesorectal excision (TME) radically improved the surgical treatment and outcome of rectal cancer. The TME principle involves *en-bloc* removal of the diseased rectum and surrounding mesorectum within an intact mesorectal fascia. Dissection in the 'holy plane' along the mesorectal fascia is said to enable preservation of the autonomic nerves.[1] Tumour involvement of the circumferential resection margin and incomplete mesorectal excision are the most important predictors for recurrent disease, emphasizing the importance of *en-bloc* removal of

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an intact mesorectal package with no disruptions, tears and/or perforations.[2,3] The Dutch TME trial showed that surgical damage to the autonomic nerves was the major cause of post-operative anorectal and urogenital dysfunction.[4,5] As a consequence, posterior, lateral and anterior dissection planes were defined in TME to warrant complete removal of the mesorectum as well as identification and preservation of the autonomic nerves.[6–9]

For such an important area, it is perhaps surprising that the precise relationships between the autonomic nerves and peri-rectal fasciae, which form the dissection planes for rectal surgeons, are still debated.[10] Denonvilliers' fascia (DVF) has been frequently studied and so far there is no consensus on its embryological origin and topological anatomy. Some have argued that DVF was more closely related to the prostate,[6,7] whilst others believed that DVF was situated closer to the rectum[11] or was even adherent with the anterior mesorectum.[12] Due to these contrary descriptions, the anterior plane in TME is frequently disputed. Some advocate dissection posterior to DVF,[13,14] some argue to 'split the layers' of DVF,[15,16] whereas others are convinced that optimal TME should be performed anterior to DVF.[12,17–19] Contradictory views on the anterior dissection plane in TME risk suboptimal surgery and consequent poorer oncologic and functional outcome. Tumour involvement of the circumferential resection margin is most frequently reported in case of anterior tumours, which are anatomically closely related to DVF.[20] In addition, incomplete mesorectal excisions are often reported.[21] Patients requiring an abdominoperineal excision (APE) of the rectum and anus suffer from a poorer oncologic outcome as low rectal tumours are technically more difficult to resect. Specifically in advanced tumours, APE has an increased risk of tumour involvement of the circumferential resection margin in comparison with anterior resection specimen.[22,23]

Excellent knowledge of the complex pelvic anatomy is a prerequisite to optimize the oncological and functional outcome of rectal cancer. However, to what extent can we enhance the outcomes in TME if the anterior plane is still questioned? We aimed to study DVF in whole mount microscopic sections of adult pelvic exenteration specimens and concentrated on the morphological and topological anatomy, the anterior plane in TME and the lateral edges in relation to the autonomic nerves and mesorectal fascia. Additionally, a developmental series of human female and male fetuses was analysed to gain better insight in the development of endopelvic fasciae.

Methods

Adult cadaveric specimens

Four human adult pelvic exenteration specimens were obtained through the University of Leeds GIFT Tissue Research Programme (www.gift.leeds.ac.uk) from consented donor bodies belonging to two males and two females. Ethical approval was granted by the Northern and Yorkshire Regional Ethical Committee, Jarrow, UK (unique reference number 11/H0903/6). There was no history of pelvic surgery or pelvic pathology at post mortem examination. The specimens were retrieved during a tissue donation autopsy performed at St. James's University Hospital in Leeds in with the body in the prone jack-knife position according to the extralevator APE technique as described by Holm et al.[24] The specimens were essentially pelvic exenterations including the anal canal and rectum up to the recto-sigmoid junction, anal sphincters, perineal body, mesorectum with an intact mesorectal fascia, levator ani muscle, obturator internus muscle, vagina or prostate and penile bulb and posterior bladder wall. After fixation in 8% formaldehyde solution for seven days, the specimens were transversely sectioned at one centimetre. The slices were photographed and dissected to fit in Super Mega Cassettes measuring 74.8 × 52.5 × 16.5 mm (CellPath; Powys; UK). All tissues were dehydrated in graded ethanol and embedded in paraffin mega blocks.

In addition, a developmental series of seven human female fetal pelvic specimens (embryonic age of 10, 12, 14, 15, 16, 19, and 20 weeks) and four human male fetal pelvic specimens (embryonic age of 9, 10, 12 and 20 weeks) were studied from collections in the Department of Anatomy & Embryology, Leiden University Medical Centre and the University of Warsaw, Poland. All fetuses were obtained with informed consent after miscarriage or legal abortion and were free of congenital pelvic malformations. The fetal specimens were dehydrated in graded ethanol and xylene, and embedded in paraffin blocks.

Histological staining

The mega blocks of the pelvic exenteration specimens were transversally cut in serial 5 μm sections. In one male and one female specimen every 10th section was collected onto glass slides and stained with haematoxylin and eosin (H&E), creating a series with a cross-sectional interval of 50 μm. Additional sections were collected from each mega block and stained with Masson's trichrome (MT) and Millers' elastin (ME).[25] In the other male and female specimen every 48th, 49th and 50th section were collected, creating three series with a cross-sectional interval of 250 μm, of which one series was stained with H&E and one series with MT. The remaining series was kept for additional stains with ME.

The paraffin blocks containing the fetal pelvic specimens were serially cut in transverse sections of 8 and 10 μm and alternately stained with H&E and azan to reveal collagen. A series was stained using antibodies against alpha-smooth muscle actin (SMA; Sigma-Aldrich, A-2547) and S-100 (S100; DAKO, Z-031101) to detect smooth muscle fibres and the peripheral neural network, respectively. The protocol used can be explored online at: www.caskanatomy.info/research.