Irish Journal of Medical Science (1971 -) (2019) 188:987–992
https://doi.org/10.1007/s11845-018-1940-9

ORIGINAL ARTICLE

Transmission electron microscopy and histological analysis of the peridural membrane

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Received: 25 September 2018 / Accepted: 19 November 2018 / Published online: 15 December 2018

Abstract

Introduction While first described in 1904, the characterisation of the peridural membrane, which is frequently encountered, yet usually unnoticed, during lumbar decompression surgery, remains inconclusive. This relatively little known membrane is continuous with the posterior longitudinal ligament and lines the epidural space. In this study, we are comparing the membrane and ligamentum flavum from patients to analyse the variations of the histological and ultrastructural compositions.

Materials and methods We took samples of the membrane and ligamentum flavum from five separate patients who were undergoing lumbar spine decompression surgery for herniated discs which were then analysed with transmission electron microscopy and stained with H&E (morphology), trichrome (collagen content), and Verhoeff-Van Gieson (elastin content).

Results Upon analysis of the peridural membrane, we observed tightly packed collagen fibres, interspaced with elastin fibres and very few fibroblasts. While the ligamentum flavum showed a significantly higher elastin to collagen ratio and looser arrangement of collagen fibres with a larger extracellular matrix. The peridural membrane was similar in appearance and constituent parts to the dura mater.

Conclusion The peridural membrane is a distinctive and important membrane in the spinal canal, and given its high collagen to elastin ratio and it tightly packed nature, we conclude that it forms a protective layer around the spinal cord which may help in minimising the compressive nature of intervertebral disc herniation.

Keywords Epidural membrane · Meningeal membranes · Peridural membrane · Posterior longitudinal ligament · Spinal surgery

Introduction

The existence of the peridural membrane (PM) has long been debated, since it was first described by Fick et al. [1]; its makeup and function have been surrounded in controversy. The literature tells us that the PM is a fibrovascular sheath about one fourth the toughness of the dura mater that extends entirely around the bony canal [2–4]. Wiltse et al. [3, 4] show that the PM inserts to the anteromedial surface of the deep posterior longitudinal ligament (PLL). It continues along the medial surfaces of the pedicles, around the caudal laminae and ligamentum flavum. At the level of the disc, it is continuous with connective tissue surrounding the radicular nerves, which Kikuchi et al. [5, 6] called it the "epiradicular sheath" and Frykholm [7] called it the "periradicular sheath". The PM is not present along the posterior surface of the intervertebral discs [4]. The veins of Batson's plexus run along the posterior surface of the PM, although these vessels often penetrate the membrane in the midline to become the basivertebral veins [3, 4]. We encountered the peridural membrane on multiple occasions in patients undergoing lumbar spine decompression/discectomy surgeries which caused some confusion during the procedure as it is rarely described in anatomical/surgical textbooks. Our aim of the study is to show the peridural membrane as a distinct anatomical layer different to the ligamentum flavum and to highlight its existence. Having to remove the material to continue with the decompression, we decided to analyse a portion of the samples to further understand the makeup of the membrane.

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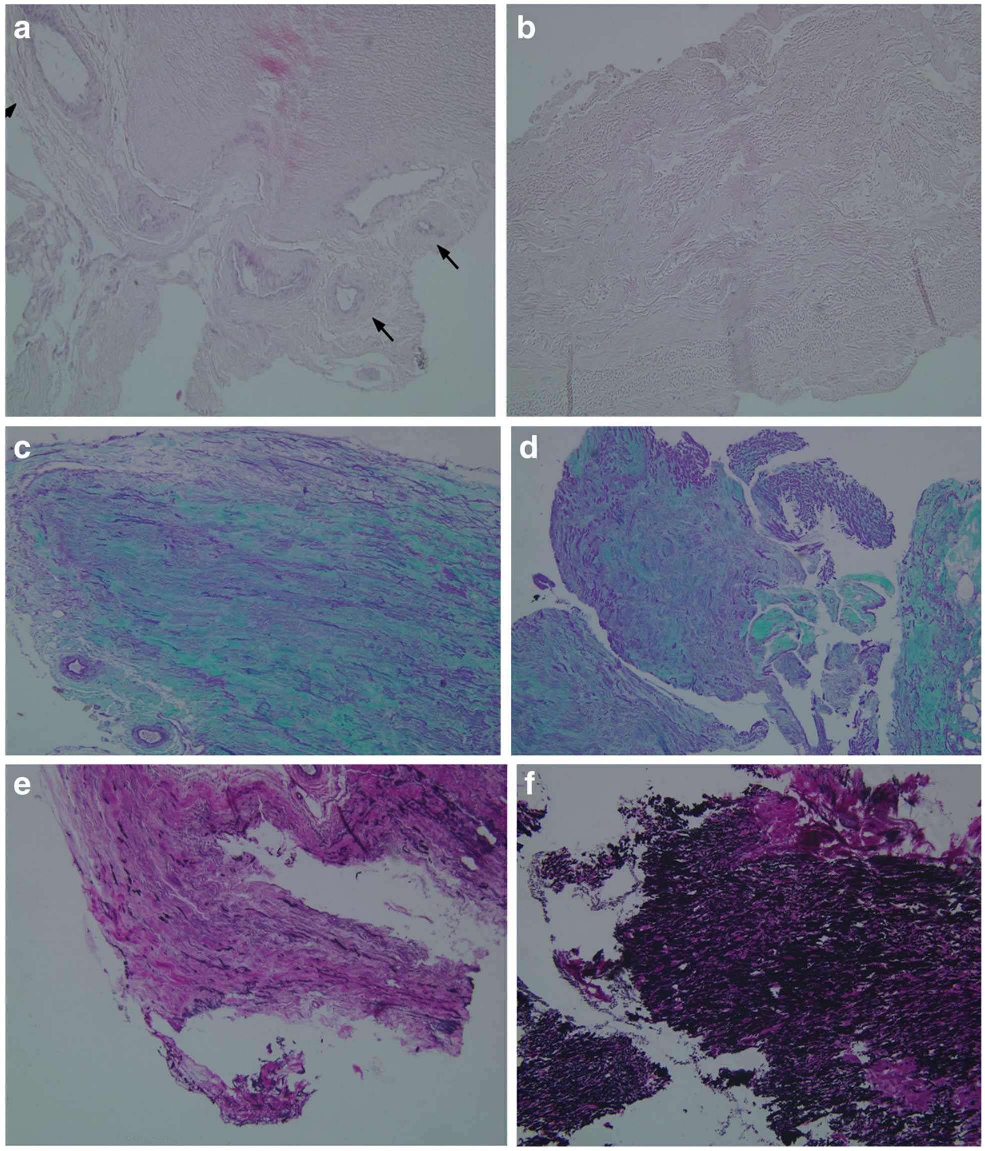


Fig. 1 H+E staining: Light microscopy showing PM (**a**) and LF (**b**), magnification × 4. Arrows show vascular and relative acellular nature of the PM compared to the ligamentum. Trichrome staining: Light microscopy showing the high collagen content of the PM (**c**) in contrast to the LF (**d**) (light blue staining), magnification × 4. Van Gieson's staining: Light microscopy showing the high elastin content of ligamentum (**f**) (black stain) compared to that of the PM (**e**), magnification × 4

Materials and methods

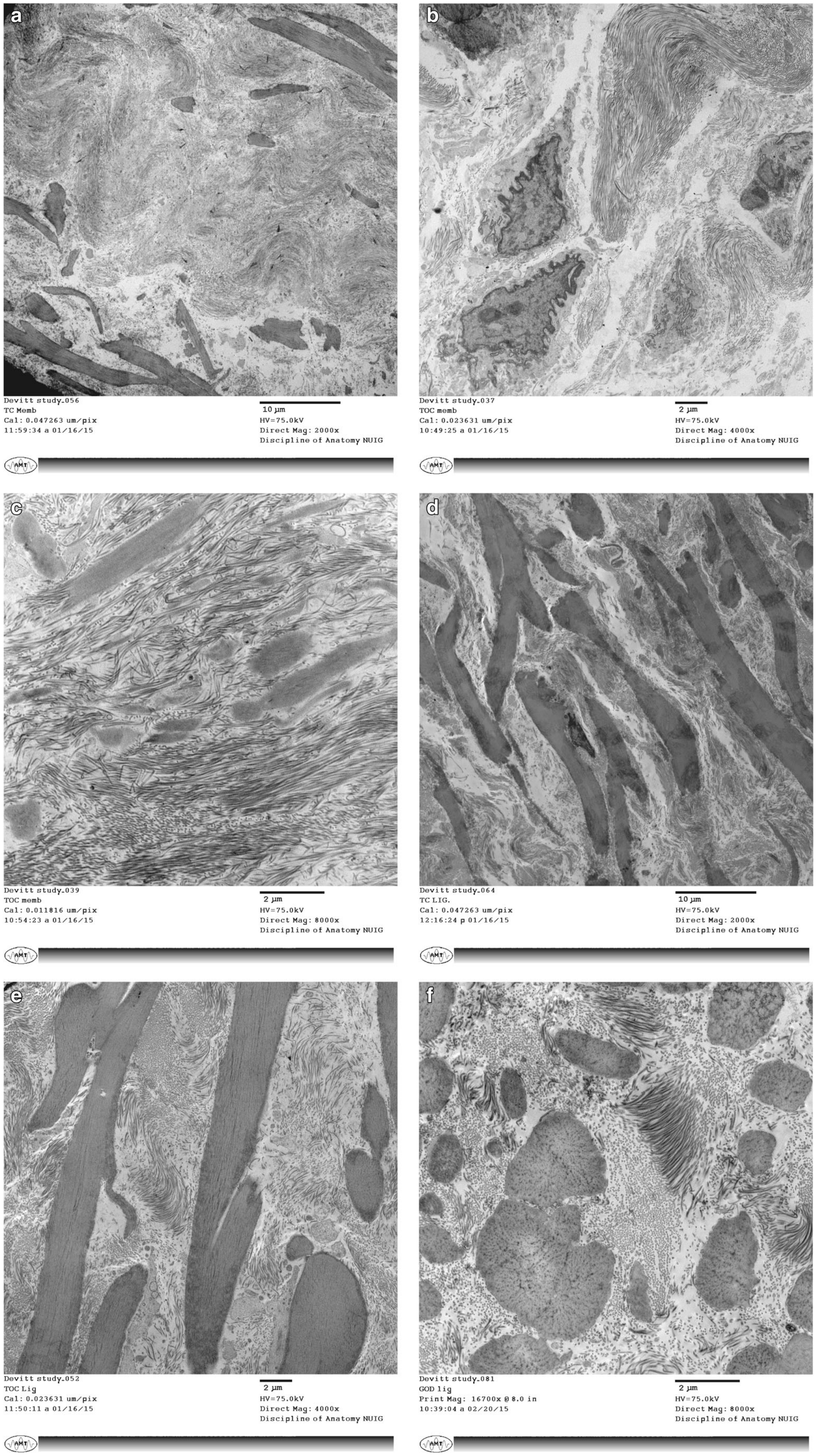
Five samples of the peridural membrane and ligamentum flavum from five patients undergoing lumbar decompression/discectomy surgeries at L4/5 level were taken and analysed using transmission electron microscopy and light microscopy. Once extracted, the specimens were placed in a 10% formalin solution until further analysis could take place. The dura mater was not violated in any of the surgeries performed. The samples were then divided in two and prepared for transmission electron microscopy (TEM) analysis or histology.

The TEM samples were then prepared using our local protocol—primary fixative, 2% glutaraldehyde + 2% paraformaldehyde in 0.1 M sodium cacodylate/HCl buffer pH 7.2; secondary fixative, 1% osmium tetroxide in 0.1 M sodium cacodylate/HCl buffer pH 7.2; dehydration with graded alcohols (50%, 70%, 90%, 95%, 100%), propylene oxide (two changes), and finally an Epson-based resin. Relevant regions of interest are selected for subsequent trimming, and ultrathin sections of the magnitude of 80–100 nm are cut and lifted onto 3-mm copper grids.

The histology samples were embedded in paraffin and cut to into 5-μm sections and then stained using H+E, Masson trichrome, and Van Gieson staining using our local protocols (see Appendices 1, 2,and 3).

The area of elastin in the TEM samples was measured using ImageJ (Version 1.47v, National Institutes of Health,

Fig. 2 Transmission electron microscopy. TEM imaging of the PM (**a** × 2000, **b** × 4000, and **c** × 8000) showing the densely packed collagen fibres with small islands of elastin and ligamentum flavum (**d** × 2000, **e** × 4000, and **f** × 8000) showing large elastin fibres with loosely packed collagen fibres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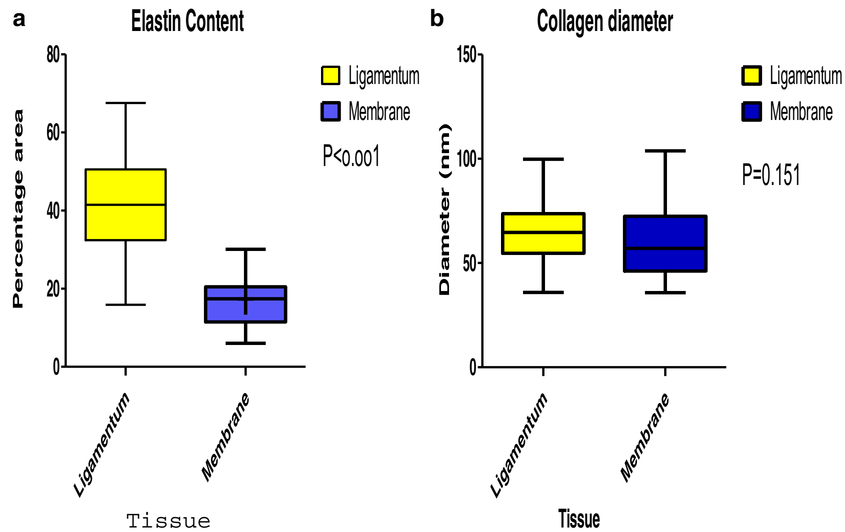

Fig. 3 **a** Statistically significant difference in percentage area of elastin content of ligamentum flavum compared to the elastin content of peridural membrane, analysed using Student's *t* test ($P < 0.001$). **b** Diameter of collagen fibres found in ligamentum flavum compared to the collagen diameter in the peridural membrane. No statistical significance (significance taken with a P value < 0.05, $n = 5$)

USA). Ten histological images were analysed for each sample; the area of elastin was calculated for each by ImageJ with the images set to binary and the elastin highlighted. Results were analysed using GraphPad™ Prism (version 5, GraphPad Software, CA, USA). Student's *t* test with a P value of < 0.05 is taken as statistically significant.

Ethical approval was granted by the Galway University Hospital Ethics Committee (reference no. C.A. 1281).

Results

Histological analysis of the samples shows a higher elastin content in the ligamentum in comparison to that in the peridural membrane. The membrane is relatively acellular in comparison but is more vascular than the ligamentum. The collagen fibres in the membrane are more organised and regulated in the membrane and disorganised in the ligamentum. Arterioles and venules were evident throughout the peridural membrane but not in the ligamentum flavum (Fig. 1).

TEM analysis shows a much organised peridural membrane with collagen fibres compacted together with small islands of elastin in contrast to the disorganised ligamentum with the fibres widely spaced. The elastin content was also analysed showing a higher ratio in the ligamentum to the membrane (Fig. 2. The diameter of the collagen fibres showed no statistical difference between the samples (Fig. 3).

Discussion

Our histological and TEM analysis of the peridural membrane shows it is a distinct, well-organised, and vascular membrane. Spinal surgeons have long undervalued the significance of the PM and may not know its existence even though they would have encountered it regularly and known even less of its many potential important functions. It has been well documented in regard to intervertebral disc herniation that it imparts a protective function, along with the posterior longitudinal ligament, for the spinal cord in preventing the nucleus pulposus from causing Cauda equina syndrome [8, 9]. It can also have implications for our anaesthetic colleagues resulting in suboptimal spinal anaesthesia [10]. Its role in lower back pain is also debatable, but a recent study by Bosscher et al. [11] shows the presence of nociceptive nerve fibres in the PM.

Compliance with ethical standards

Ethical approval was granted by the Galway University Hospital Ethics Committee.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

Appendix 1

Haematoxylin and eosin:

1. Dewax sections in xylene; two changes of 10 min each; this is carried out in the fume hood
2. Remove xylene: two changes of absolute alcohol 2 min each
3. Bring to water through 95%, 70%, and 50% alcohols, 2 min each bath
4. Remove alcohol in running tap water, 2 min
5. Stain in Mayer's haematoxylin 6 min

6. Blue nuclei in running tap water for 4 min
7. Examine under microscope and differentiate in acid alcohol if necessary
8. If acid alcohol used, blue nuclei in running tap water for 4 min
9. Stain in eosin for 2 min
10. Rinse in tap water quickly
11. Dehydrate through 50% alcohol 10 s
12. Dehydrate through 70% alcohol 10 s
13. Dehydrate through 90% alcohol 2 min
14. Dehydrate through absolute alcohol 2 min × 2
15. Clear in xylene, two changes of 10 min
16. Cover sections with DPX mounting medium and apply coverslip
17. Place slide in oven to allow mounting medium to solidify

Results:

Nuclei stained dark blue
Cytoplasm stained pink

Appendix 2

Masson's trichrome with Gomori's aldehyde fuchsin:

1. Dewax sections in Histoclear; two changes of 10 min each
2. Remove xylene
3. Two changes of absolute alcohol 1 min each
4. Bring to water through 95%, 70%, and 50% alcohols, 1 min each bath
5. Remove alcohol in running tap water, 2 min
6. Oxidise in 0.5% $K_{mn}O_4$/0.5% H2SO4 (equal parts) 2 min
7. Rinse in tap water and bleach in 2% sodium metabisulphite ($Na_2S_2O_5$), 2 min
8. Wash in water 30 s, followed by 70% alcohol, 1 min
9. Stain in Gomori's aldehyde fuchsin, 1 min
10. Rinse in water (very quickly) then 95% alcohol 10 s, then water again 10 s
11. Stain in Celestine blue, 4 min
12. Rinse in water, 30 s
13. Stain in Mayer's Haemalum, 4 min
14. Quick rinse in water (20 s)
15. Differentiate in acid alcohol, 20 s
16. Blue nuclei in running tap water, 4 min
17. Stain in Masson's cytoplasmic stain, 1 min
18. Rinse very quickly in water and differentiate in 1% dodeca-molybdophosphoric acid (H_3PO_4 12 MoO_3 24 H_2O), 2 min
19. Rinse in water and counterstain in fast green or light green, 1 min
20. Differentiate in 1% acetic acid, 1 min
21. Dehydrate through 50%, 70%, and 95% alcohols and absolute 1 min each bath
22. Clear in xylene, two changes 10 min
23. Mount in DPX (in the fume hood)

Results:

Nuclei—blue/black
Muscle—red
Collagen—green
Epithelium—purple
Keratin—red
Goblet cells—purple with green mucin

Appendix 3

Verhoeff-Van Gieson staining protocol for elastic fibres:

1. Dewax sections in Histoclear; two changes of 10 min each
2. Remove xylene
3. Two changes of absolute alcohol 1 min each
4. Bring to water through 95%, 70%, and 50% alcohols, 1 min each bath
5. Remove alcohol in running tap water, 2 min
6. Stain in Verhoeff's solution (5% alcoholic haematoxylin, 10% ferric chloride, Weigert's iodine solution) for 1 h
7. Rinse in tap water with two to three changes
8. Differentiate in 2% ferric chloride for 1–2 min
9. Stop differentiation with several changes of tap water
10. Wash slides in tap water
11. Treat with 5% sodium thiosulfate for 1 min. Discard solution
12. Wash in running tap water for 5 min
13. Counterstain in Van Gieson's solution for 3–5 min
14. Dehydrate through 50%, 70%, and 95% alcohols and absolute 1 min each bath
15. Clear in xylene 2 changes 10 min
16. Mount in DPX (in the fume hood)

Results:

Elastic fibres—blue-black to black
Nuclei—blue to black
Collagen—red
Other tissue elements—yellow

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