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ORIGINAL ARTICLE

The morphological and microscopical characteristics of posterior layer of human thoracolumbar fascia; A potential source of low back pain

Aspects morphologiques macroscopiques et microscopiques de la couche postérieure du fascia thoracolombaire chez l'homme

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KEYWORDS

Deep fascia;
Thoracolumbar fascia;
Peripheral nerve endings;
Nociceptors;
Low back pain;
Angulation;
Collagen fibers;
Morphology;
Morphometry

Summary

Background. – Posterior layer of thoracolumbar fascia (PTLF) is the deep fascia of back of the trunk, which connects the trunk, upper limb and lower limb muscles. Very few cadaveric studies of posterior layer of thoracolumbar fascia (PTLF) are found in the literature, which mention the presence of nerve receptors in it but, quantification of the nerve receptors where not found. Providing the morphological and morphometrical data of PTLF may help the exercise physiologists, sports physicians, occupational health assistants and, physiotherapists to modify or invent new protocol of treatment to help the society.

Methods. – In this study, twenty formalin embalmed human cadavers were used and we have documented the orientation of the PTLF and quantified the number of peripheral nerve endings at the different vertebral levels.

Results. – Mean distance of PTLF from vertebral spines to the musculofascial junction was at thoracic region 3.38 cm and 3.34 cm; at lumbar region, it was 7.4 cm and 7.36 cm and at sacral region it was 2.98 cm and 2.96 cm on right and left side, respectively. The angulation of PTLF varies from 18–110 degrees at different vertebral levels. The microscopic data shows the thickness of PTLF and number of nerve endings in the sacral level is increased compared to that of thoracic vertebral levels.

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S. Marpalli, K.G. Mohandas Rao, P. Venkatesan et al.

Conclusions. – We have contributed the novel morphological and microscopical details to the limited existing data on PTLF. We also have provided the quantitative data of nerve fibers, which are possible nociceptors of PTLF.

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Résumé Dans cette étude, vingt cadavres humains embaumés au formol ont été utilisés et nous avons analysé l'orientation du PTLF et quantifié le nombre de terminaisons nerveuses périphériques aux différents étages vertébraux. La distance moyenne du PTLF des épines vertébrales à la jonction musculofasciale était au niveau de la région thoracique de 3,38 cm et 3,34 cm ; dans la région lombaire, elle était de 7,4 cm et 7,36 cm et dans la région sacrée de 2,98 cm et 2,96 cm respectivement sur le côté droit et gauche. L'angulation du PTLF variait de 18 à 110 degrés à différents étages vertébraux. Les données microscopiques montrent que l'épaisseur du PTLF et le nombre de terminaisons nerveuses dans la région sacrée est augmenté par rapport à celui des étages vertébraux thoraciques. Nous apportons de nouveaux détails morphologiques et microscopiques aux données existantes limitées sur le PTLF. Nous avons également fourni les données quantitatives des fibres nerveuses, qui sont des nocicepteurs possibles du PTLF.

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Introduction

Thoracolumbar fascia (TLF) is the major fascial connection between the trunk, upper limb and lower limb muscles. TLF plays main role in transmitting forces from the lower to the upper limbs and vice versa [1]. The thoracolumbar fascia has three layers, namely posterior, middle and anterior layers (Fig. 1A). The posterior layer of thoracolumbar fascia (PTLF) is attached medially to lumbar and sacral spines and adjoining supraspinous ligaments, below to the iliac crest and above to the lower border of 12th rib and to the lumbo-costal ligament [2]. With gluteus maximus, latissimus dorsi and lower fibers of trapezius muscles attached to it (Fig. 1D), the PTLF forms the musculofascial structure which acts as myofascial linkage and controls, facilitates and transfers the load for proper functioning of the body [1]. The PTLF is made up of 2 layers (Fig. 1B and C). The outer layer or PTLF is made up of a superficial lamina of parallel collagen fibers, major part of which is contributed by the aponeurosis of the latissimus dorsi and serratus posterior inferior muscles and a deep lamina consisting of a reticular sheath which encloses the paraspinal muscles [3].

According to recent studies, cases of low back pain are on the rise and it causes more global disability than any other conditions. In the past, the diagnostic procedures to identify and treat disc pathologies and joint problems were always given prime importance in cases of low back pain, whereas the TLF was largely ignored as a potential source of low back pain (LBP) [4–6]. Recent studies on innervation of TLF indicate that the fascia exhibits a clear nociceptive neural capacity and therefore may be a potential source of pain in some cases of low back pain, which is so far named as ‘‘nonspecific low back pain’’ [7,8]. The anatomical study of TLF caught attention of investigators because of its probable nociceptive capacity [5,8]. Histological studies of TLF revealed string of pearl appearance of free nerve endings

[9]. These fibers are the branches of dorsal rami of spinal nerves [5]. In vivo studies with experimental elicitation of LBP by stimulating TLF suggest an increased sensitivity of dorsal horn neurons, thus TLF could possibly be a frequent source of low back pain [7]. Micro-injuries of TLF may result in low back pain and further biomechanical impairments [10]. Though many researchers found the dorsal rami of spinal nerves (cluneal nerves) piercing through TLF [5], quantification of the same and the regional differences in their occurrence, which could be decisive in planning the low back pain management, were not found in the literature. Attachment of TLF, collagen fiber directions, size, thickness and biomechanical properties of TLF have been documented by earlier workers [9–11]. However, a surface anatomy model of PTLF with, quantitative measurement of angulation of its fibers in relation to the bony landmarks such as vertebral spines, the exact measurement of PTLF from vertebral spines to its musculofascial junction and the thickness of the superficial lamina of PTLF has not been established. We propose the establishment of comprehensive documentation of all the anatomical facts of PTLF. The present paper therefore aimed to document the morphological and microscopic anatomy of PTLF.

Materials and methods

Institutional ethical committee approval Registration No. ECR/146/Inst/KA/2013/RR16 was taken prior to the study.

Human cadavers

Twenty formalin embalmed healthy looking adult human cadavers aged between 50–75 years (mean age 65 years) were obtained from the Department of Anatomy, Melaka

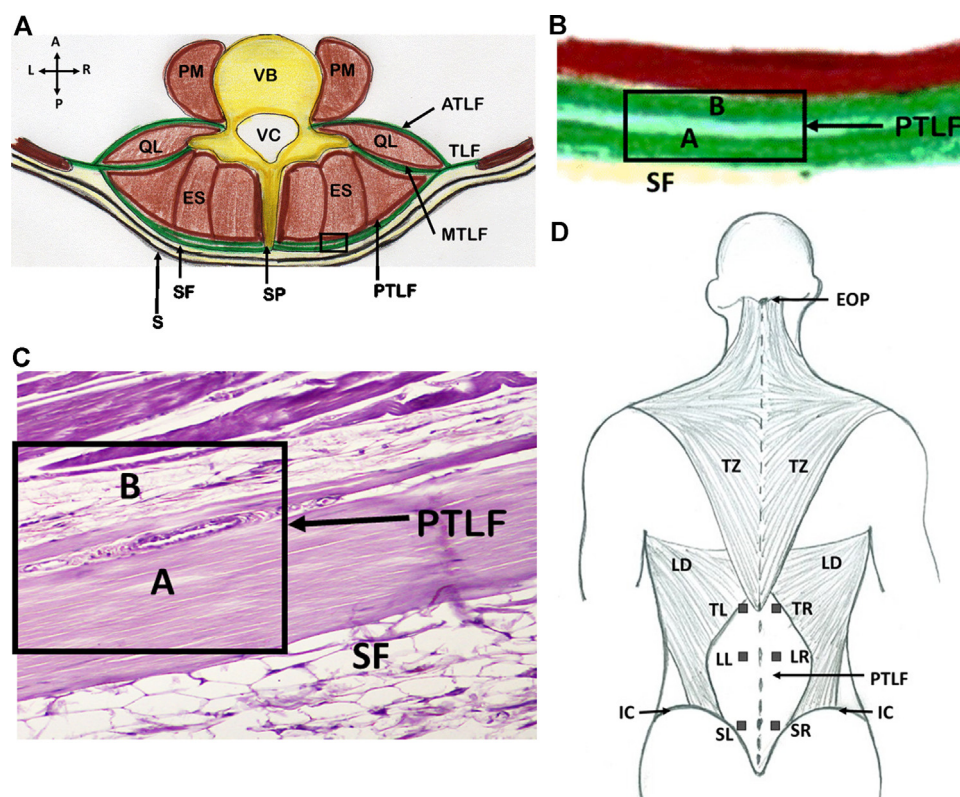


Figure 1 A. Diagrammatic representation of transverse section of back of the trunk showing the layers of thoracolumbar fascia (TLF) and muscles enclosed by its layers. The site of tissue collection for the present study has been diagrammatically indicated by a rectangle block. B. Diagrammatic representation of transverse section through the posterior layer of thoracolumbar fascia (PTLF) showing its superficial (A) and deep (B) laminae. C. Photomicrograph of transverse section through posterior layer of thoracolumbar fascia (PTLF) showing its superficial (A) and deep (B) laminae. S: skin; SF: superficial fascia; MTLF: middle layer of thoracolumbar fascia; ATLF: posterior layer of thoracolumbar fascia; SP: spinous process; VB: vertebral body; VC: vertebral canal; QL: quadratus lumborum muscle; ES: erector spinae muscle; PM: psoas major muscle. D. Diagrammatic representation of the back of the trunk and posterior layer of TLF showing the 6 sites of tissue collection. Small blocks indicate the sites of tissue piece (1 cm × 0.5 cm) collection on right (R) and left (L) sides, 3 cm lateral to the spinous process of the T12, L2, and S1 vertebrae. TR: thoracic right; TL: thoracic left; LR: lumbar right; LL: lumbar left; SR: sacral right; SL: sacral left; PTLF: posterior layer of thoracolumbar fascia; IC: iliac crest; LD: latissimus dorsi muscle; TZ: trapezius muscle.

Manipal Medical College, Manipal Academy of Higher Education, Manipal, India. All twenty cadavers were used for gross morphological and morphometric study of PTLF and ten randomly selected cadavers from them were used for the microscopic structural study of the PTLF.

Morphological and morphometric study of PTLF

The skin of the back of the trunk of 20 adult cadavers were reflected as per the guidelines given in the Cunningham's manual of practical Anatomy [12]. To record the accurate size and extent of PTLF, the superficial tissue zones were cleaned and measurements were taken from the 12th thoracic to 3rd sacral vertebral spines to the musculofascial junctions of PTLF by using metal metric ruler (accuracy ± 1 mm). The spines of the vertebral column are considered as the midline and the reference point to measure the angles. The directions of fibers (angulations) in PTLF were analyzed manually in the cadaver using international

standard goniometer (accuracy ± 1) [13]. Posterior surface of the fascia was photographed in situ using Nikon (Japan) 5100 digital camera without any special effects.

Microscopic study of PTLF

Tissue collection

The PTLF from 10 formalin embalmed cadavers were used for the study. Before collecting the specimens, it was ensured that there was no gross pathology or previous surgical procedures performed or traumatic damage to the back of any of the cadavers. After reflecting the skin of the back of the trunk as per the guidelines given in the Cunningham's manual of practical Anatomy [12], three regions of the PTLF were selected for collection of specimens. Pieces of PTLF measuring about 1 cm × 0.5 cm × 0.5 cm were removed at the level of 12th thoracic vertebra (T12), 2nd lumbar vertebra (L2) and 1st sacral vertebra (S1) on right and left sides from the regions 3 cm lateral to the corresponding spinous

processes. These pieces were identified as thoracic right (TR) and thoracic left (TL), lumbar right (LR) and lumbar left (LL), sacral right (SR) and sacral left (SL) (Fig. 1A–D).

Hematoxylin and eosin staining

The samples were mounted on a cardboard to avoid any deformation, formation of artefacts and were fixed in 10% buffered formalin (pH 7.4) for 24 hours. Samples were then dehydrated in ethanol, defatted in xylene and fixed in paraffin. Histological procedures were uniform for all the sections. Series of sections were taken using a rotary microtome at 7 micron thickness and stained with eosin and hematoxylin stain as per the standard protocol. Stained tissue slides were then observed under Olympus BX43 microscope (USA) attached with DP 21 microscope digital camera (Japan). Photographic images were captured under $20\times/40\times$ magnification. These images were then analyzed for quantification of peripheral nerve endings and nociceptors [13]. The thickness of the superficial lamina of PTLF (accuracy $\pm 1\ \mu\text{m}$) was measured using the image-J software [14]. The quantification of the peripheral nerves were done using Magnus Mlx. Dx 220-240V-0.2A50HZ Olympus (INDIA) Pvt. Ltd. microscope with the help of ocular micrometer [15]. Peripheral nerve endings were counted within a specific area in the deep lamina of posterior layer of thoracolumbar fascia.

Stereological cell counting

As mentioned earlier, tissues were collected from 10 cadavers and from each cadaver, 6 tissue pieces (from regions TR, TL, LR, LL, SR, SL) were taken. From each of this tissue piece, 6 serial sections with the interval of 10 sections were used for the structural quantification, stained sections were then observed under microscope and deep lamina of PTLF were considered for quantification of peripheral nerve endings and nociceptors. The pain receptors in the superficial lamina was difficult to count because of densely packed collagen fibers. From each section, a minimum of three fields and total of 18 fields were considered for each region. Connective tissue and blood vessels were also identified [16].

Statistical analysis

The quantitative data presented as mean \pm standard error of mean and was analyzed using the SPSS software package 16.0. For comparison between the groups, One way Anova followed by Tukey's test was used. The P -value < 0.05 was considered as statistically significant.

Results

Morphological and morphometric observations of PTLF

It has been noted that the width of PTLF from posterior midline of the trunk (vertebral spine) to the musculofascial junction at different vertebral level were different. At the level of 4th lumbar (L4) vertebra, it is broader ($7.4 \pm 0.23\ \text{cm}$) compared to the other vertebral levels. At 3rd sacral vertebral level (S3), it is very narrow measuring

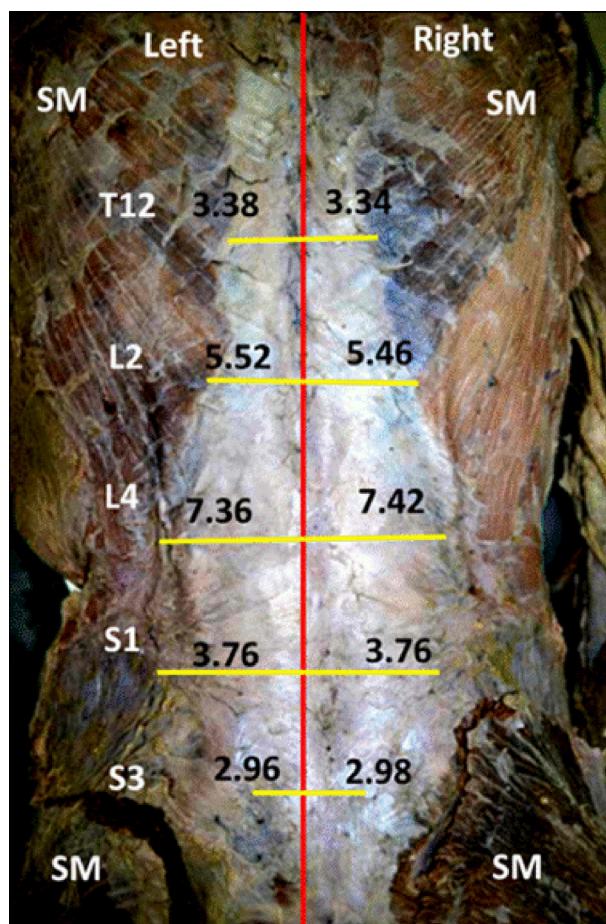


Figure 2 Shows the posterior surface of posterior layer of thoracolumbar fascia (PTLF). Whitish shining area marked with red arrows indicates the PTLF and brownish area marked SM indicates the skeletal muscles attached to PTLF. Transverse yellow lines indicate the distance from corresponding vertebral spines (T12, L2, L4, S1 and S3) to the musculofascial junction of posterior layer of TLF. Red vertical line indicates the midline (vertebral spines).

about $2.98 \pm 0.08\ \text{cm}$ (Fig. 2). The measurements of right and left sides at the same vertebral level was found similar and is shown in the Table 1 and there was no significant difference in the breadth of PTLF on right and left sides. The angulation (fibre orientation) of PTLF at the different vertebral levels was found significantly different in different cadavers (Table 2). The mean angulation of PTLF at thoracic and lumbar levels were found within the range of 70–90 degrees, but at the level of S3 vertebra, the angulation was about 18–20 degrees lesser (Fig. 3A and B, Table 2).

Microscopic study of PTLF

The thickness of superficial lamina of PTLF in the sacral vertebral level (S1) is significantly ($P < 0.01$) thicker when compared to that of thoracic vertebral level (Table 3). There was not much difference in the thickness of superficial lamina of PTLF between right and left sides at a particular vertebral level. The number of peripheral nerve receptors in the deep lamina of PTLF is shown in the Table 4. The Fig. 4A

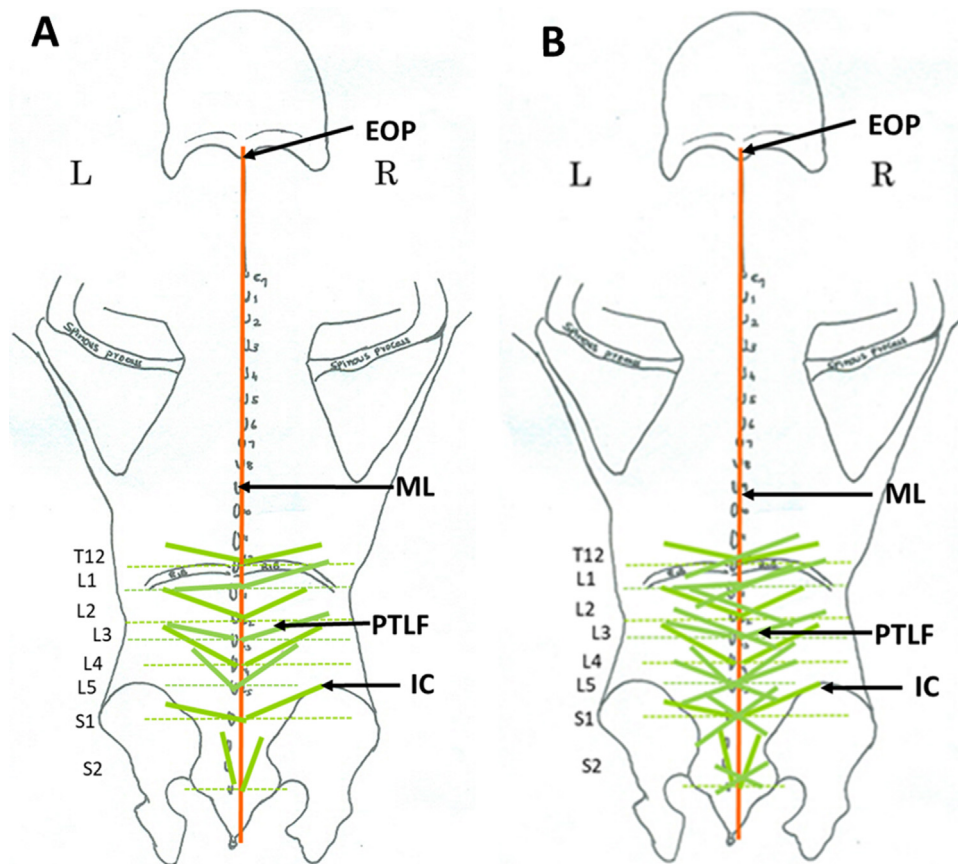


Figure 3 Schematic representation of back of the trunk. Diagram 3A shows the mean angulation of fibers of posterior layer of TLF on right (R) and left (L) sides at the levels of spines of T12 to S2 vertebrae. Diagram 3B shows the overall angulation of posterior layer of TLF on right (R) and left (L) sides at the levels of spines of T12 to S2 vertebrae.

Table 1 Values are mean [\pm standard error of mean (SEM)] distance from corresponding vertebral spines (T12, L2, L4, S1 and S3) to the musculofascial junction of posterior layer of thoracolumbar fascia (PTLF).

Vertebral level	Side	Mean \pm SEM (n = 20) distance in cm
T12	Right	3.38 \pm 0.10
	Left	3.34 \pm 0.10
L2	Right	5.48 \pm 0.47
	Left	5.52 \pm 0.52
L4	Right	7.4 \pm 0.23
	Left	7.36 \pm 0.16
S1	Right	3.76 \pm 0.14
	Left	3.76 \pm 0.15
S3	Right	2.98 \pm 0.08
	Left	2.96 \pm 0.08

shows the stained sections of PTLF at thoracic, lumbar and sacral vertebral levels. The results of post hoc turkeys test showed a significant difference ($P < 0.05$) in the mean number of peripheral nerve receptors (Fig. 4B) between the groups. At SR and SL the nerve receptors were significantly more when compared with that at TR and TL. Results showed

significant ($P < 0.001$) increase in the number of receptors at LR, SR and SL when compared with that at TR, however there was no significant difference in mean number of nerve receptors between TR with that at TL and LL.

Discussion

Morphological and morphometric observations of PTLF

Many researchers have studied the orientation of collagen fibers within the PTLF. Fiber angle were described by them was varied from horizontal superiorly to sloping craniolateral-to-caudomedial direction [17,18]. The angle formed by the meeting point of collagen fibers were progressing from a shallower angle (70–90 degrees) superiorly to a steeper angle (18–20 degrees) inferiorly. In the present study, the similar pattern is observed till the level of L3 vertebra, but the angulation found diminished thereafter till S3 vertebra. Creze et al., reported craniolateral-to-caudomedial direction of fibers in the superficial lamina of PTLF above L4 vertebral level and crosshatched appearance below it [19,20]. Present study also showed similar results, apart from that we also have reported the average angulation of fibers in PTLF at different important vertebral levels.

Table 2 Fibre orientation of posterior layer of thoracolumbar fascia in relation to the vertebral spines. Values represent the angulation (in degrees with accuracy of $\pm 1^\circ$) of the fibers of posterior layer of thoracolumbar fascia (PTLF) on right (R) and left (L) sides at the level of spines of the T12, L1, L2, L3, L4, L5, S1 and S2 vertebrae.

Vertebral level	Side	Mean angulation (in degrees) \pm SEM (n = 20) of fibers of PTLF
T12	Right	74.00 \pm 6.83
	Left	82.00 \pm 7.74
L1	Right	78.60 \pm 7.86
	Left	91.00 \pm 6.76
L2	Right	88.20 \pm 8.13
	Left	85.20 \pm 5.78
L3	Right	89.20 \pm 6.81
	Left	86.60 \pm 2.47
L4	Right	73.40 \pm 4.85
	Left	74.60 \pm 3.76
L5	Right	68.60 \pm 2.42
	Left	71.60 \pm 5.40
S1	Right	53.00 \pm 1.06
	Left	72.80 \pm 5.54
S2	Right	18.00 \pm 2.00
	Left	19.80 \pm 1.21

Table 3 Values are mean [\pm standard error of mean (SEM)] thickness of posterior layer of thoracolumbar fascia (PTLF) at thoracic, lumbar, sacral vertebral levels on right and left sides.

Sections of PTLF at different vertebral levels	Mean thickness of PTLF in $\mu \pm$ SEM (n = 10)
Thoracic right (TR)	192.87 \pm 4.11
Thoracic left (TL)	181.87 \pm 1.05
Lumbar right (LR)	243.67 \pm 1.32
Lumbar left (LL)	241.77 \pm 3.49
Sacral right (SR)	428.67 \pm 8.318
Sacral left (SL)	433.54 \pm 5.28

Table 4 Values are mean [\pm standard error of mean (SEM)] of peripheral nerve receptors in the deep lamina of posterior layer of thoracolumbar fascia (PTLF) at thoracic, lumbar, sacral vertebral levels on right and left sides.

Histology sections	Number of peripheral nerve receptors in the deep lamina of PTLF in Mean \pm SEM (n = 10)
Thoracic right (TR)	10.210 \pm 0.374
Thoracic left (TL)	11.204 \pm 0.422
Lumbar right (LR)	12.215 \pm 0.507
Lumbar left (LL)	11.95 \pm 0.457
Sacral right (SR)	12.762 \pm 0.42
Sacral left (SL)	12.48 \pm 0.236

The average angulation of fibers in sacral level is much less compared to thoracic and lumbar vertebral levels. The direction of collagen fibers is one of the anatomical factors to determine the force transmission through the PTLF. The attachment and actions of latissimus dorsi above L3 and that of gluteus maximus below it may be the reason for this differences.

Breadth of PTLF in relation to different vertebral levels to understand and palpate the PTLF was not recorded so far. Present study also provides data for surface anatomy details of PTLF, for diagnosing pathologies like taught bands, designing proper palpation techniques and executing treatment strategies.

Microscopic study of PTLF

Barker and Briggs, 1999 measured the thickness of PTLF and was found to be approximately 520–550 μm in the lumbar region and was found significantly thinner in the thoracic region [17]. Present results showed the similar pattern of progressively increasing thickness of PTLF from thoracic to sacral regions, however, the measured thickness varied from 190–450 μm . In the present study, the thickness of superficial lamina of PTLF that is the collagen bundle thickness was measured digitally to document the data more accurately. However, the earlier data available in this regard are of manual measurements and that could be one of the reasons for the variations in the values [18]. The quantitative data of thickness of superficial lamina of PTLF is being reported for the first time. Earlier reports suggest that the superficial lamina is mainly contributed by latissimus dorsi and serratus posterior inferior muscles [1]. Superficial lamina of PTLF also gives attachment to, gluteus maximus in the sacral region. There are studies suggesting increased thickness of TLF in inflammatory response or soft tissue injury. An animal study conducted recently demonstrated that a soft tissue injury induced in the lumbar region of porcine model lead to fibrosis and significant thickening of thoracolumbar fascia [21]. An ultrasound based human study concluded that the patients with chronic low back pain had 25% thickness compared to the control group [22]. These studies reinforce that the data on the normal thickness of PTLF is important in clinical practice. Digitally and accurately measured thickness chart provided by us would be helpful for further studies and diagnosis of nonspecific low back pain.

The quantification of peripheral nerves and nociceptors in posterior layer of thoracolumbar fascia has not been reported earlier. These nerve fibers were easily quantifiable in the deep lamina of PTLF. In this present study, we could identify and quantify the number of nerve fibers in the deep lamina of PTLF, which pierces the superficial lamina and appears in subcutaneous zone. Here, it was found that the nerve fibers are more in the lumbar and sacral regions compared to the thoracic region. There are number of earlier reports stating that the nociceptors in PTLF are be very sensitive to chemical stimulation and may contribute to acute and chronic low back pain [23–25]. Based on this, it is evident that the low back pain is be more in lumbar and sacral regions, in other words, the pain intensity is more in lower part of PTLF than in its upper part.

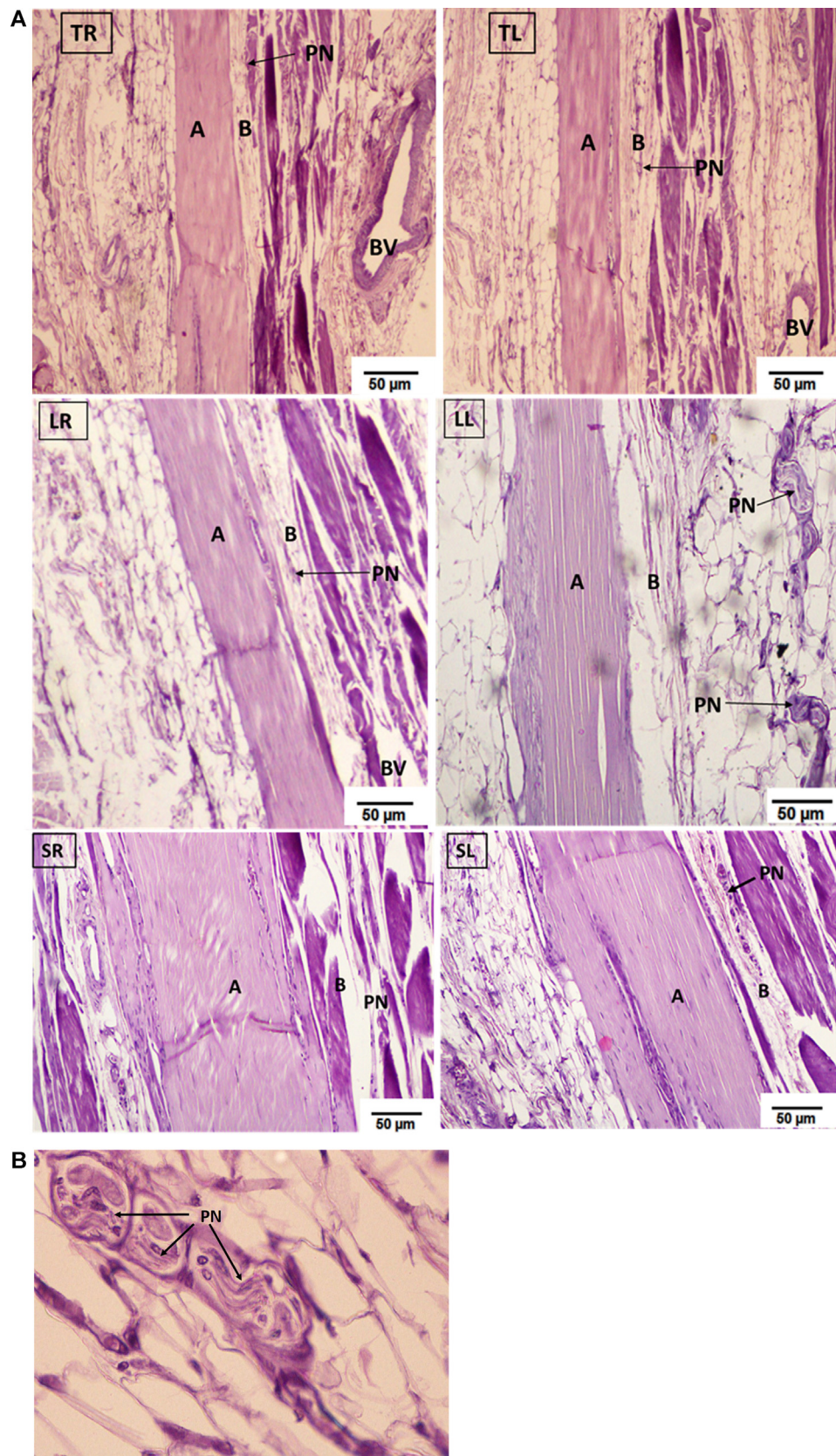


Figure 4 A. Photomicrographs of H & E stained sections of PTLF at the level of T12, L1 and S1 vertebrae showing the peripheral nerve endings, connective tissue and blood vessels. B. Magnified photomicrographs of H & E stained sections of PTLF showing the peripheral nerve endings, connective tissue and blood vessels. TR: thoracic right; TL: thoracic left; LR: lumbar right; LL: lumbar left; SR: sacral right; SL: sacral left; A: superficial lamina PTLF; B: deep lamina of PTLF; PN: peripheral nerve; BV: blood vessels.

Conclusion

The thickness of PTLF and peripheral nerve fibers in its deep lamina in the sacral region is more than in the thoracic region. The quantitative data of nerve fibers, nociceptors in the PTLF suggested that their abundance is more in the sacral region.

Role of authors

S. Marpalli: dissected the cadavers, histological sections made, helped in preparing the draft.

K.G. Mohandas Rao & P. Venkatesan: contributed in analyzing the data and drafting the article.

B.M. George: research idea given, monitored the procedures, contributed for analyzing and drafting the data.

Disclosure of interest

The authors declare that they have no competing of interest. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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