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Original Research Article

Histological morphology of ligamentum teres femoris in human cadavers

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Abstract

Background: Ligamentum teres femoris (LTF) has synovial layer, sub-synovial connective tissue and collagen bundles. LTF assists in preventing dislocation, provides mechanical stability to the hip with injury leading to joint pain and instability. The knowledge from histological structure may improve understanding of its structure and function.

Materials and Methods: Forty-four limbs, average age 70.1 ± 8.2 years, were dissected. The protocol was approved by the Institutional Ethics Committee. Those with any damaged hip, signs of previous surgery, and disarticulated limbs were excluded. The ligament was absent in one, thus, 42 specimens (right-21; left-21) were used. Tissues from two ends, and centre were stained for H&E, Masson's Trichrome, Verhoeff Van Gieson and Palmgren silver-stain. The number, diameter, and total luminal surface area of blood vessels, and number, and thickness of collagen bundles, and thickness of sub-synovial tissue were measured Image J.

Results: Synovial layer covered ligament, and collagen bundles were seen in the core. Number of blood vessels, thickness of collagen bundles and sub-synovial tissue was significantly more in middle part. Average diameter of blood vessels was significantly more in distal part and luminal surface area was in proximal, and collagen bundles number in proximal. Difference in number, diameter, luminal area of blood vessels, and number of collagen bundles between right and left sides was not statistically significant.

Conclusion: The findings support a potential nutritive role of the ligamental vessels to femur head. Nerve fibres identified in the sub-synovial area support its nociceptive role. Collagen bundles and elastic fibres suggest contribution to mechanical stability of hip joint.

Keywords: Ligamentum teres, Histology, Hip joint, Fovea capitis, Morphology

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1. Introduction

Ligamentum teres femoris (LTF), one of the ligaments supporting hip joint, extends from the cotyloid fossa of the acetabulum to the fovea on the head of femur.[1,2] It is one of the least known anatomical structures. The Hippocrates of Kos in V-IV century B.C. provided its earliest description in medical literature, where it was named as *neruus* in Latin translation. An account of ligament from III-II century BC was quoted in a text by Galen in 130-200AD.[3] The presence of LTF was indicated in the *Torah* as early as XII-II century B.C., most likely the first to explain about its damage and the subsequent disorder of the gait mechanism. An anatomist named Herophilus, from IV-III century BC, was doing cadaver dissections and was aware of an intra articular ligament in the hip joint.[3,4] In 16th century, Vesalius had made note of LTF in his work *Epitome.*[3] The name ligamentum teres is given due to its round contour, and it is believed to be first named so by Vidian in 1626.[3]

The LTF arises from the embryonic mesenchymal cells in the hip joint of foetus at two months. By the 3rd month, it encloses a few arteries and veins, supplying the fovea of femoral head.[5] The entire length of the ligament has three histological layers. The outer synovial layer covers the surface of ligament and consists of cuboidal or squamous epithelial cells. Sub-synovial layer contains loose connective tissue, adipose tissue with scattered fibroblasts, and large blood vessels. Inner dense collagen layer is composed of collagen bundles (mostly type I, III, IV), small blood vessels, scattered fibroblasts and elastic fibres.[6,7] The artery of LTF comes from the obturator artery in 80% and from the medial circumflex femoral artery in 20%.[8] The presence of blood vessels within the ligament was first reported by Paletta in

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1820.[9] Tucker described the artery of LTF in 33% infants and children, and in 70% adults, and its average diameter was 0.328 mm in the adults.[10] Wolcott (1943) found the LTF artery in 80% specimens.[11]

Recently with the increased use of arthroscopy, LTF has gained clinical attention and is believed to provide some mechanical stability to the hip joint. LTF assists in preventing dislocation in the forward and outward directions.[12] The injury to LTF has been cited as the third most common reason for hip pain in athletes,[13] and the complete tears of LTF are one of the etiological factors for hip instability. Majority of studies on LTF are from clinical settings,[12,13] and more are thus desirable on the internal structure of ligament. The knowledge from histological structure is likely to better the understanding of structure and function of the ligament. It would also be useful during reconstructive arthroscopic surgeries for hip instability due to LTF tear and fracture-dislocations of hip joint.

2. Material and Methods

This study was conducted in the department of Anatomy in the adult human cadavers over a period of two years, 2018-2020. The study protocol was duly approved by the Institutional Ethics Committee. A total of 44 limbs from 22 formalin-embalmed cadavers of either sex, average age 70.1 ± 8.2 years (range: 50 to 81 years), were dissected. The cadavers with any damage in the hip, signs of previous hip surgery, and disarticulated limbs were excluded. The ligament was absent on the left and rudimentary on the right side of a male cadaver. Thus, a total of 42 specimens (right-21; left-21) were used for histological analysis.

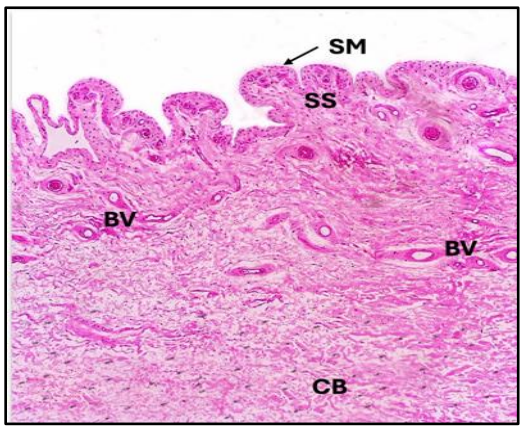

Figure 1: Transverse section of femoral end of ligamentum teres femoris showing blood vessels in the sub-synovial tissue and dense staining collagen bundles in the substance of ligament. BV: blood vessels; CB: collagen bundles; SM: synovial membrane; SS: sub-synovial tissue. Haematoxylin and eosin (4X).

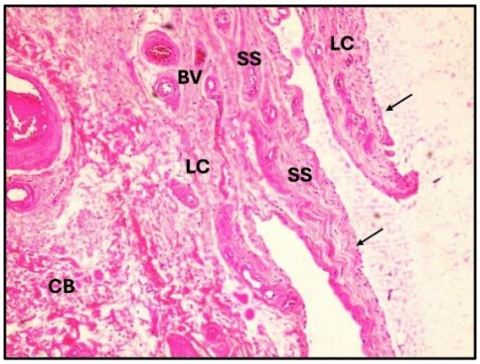

Figure 2: Transverse section of ligamentum teres femoris showing synovial folds with blood vessels and loose connective tissue. The connective tissue in synovial folds (arrows) is continuous with the rest of the sub synovial tissue. LC: loose connective tissue; BV: blood vessels; SS: sub-synovial tissue; CB: collagen bundles. Haematoxylin and eosin (10X).

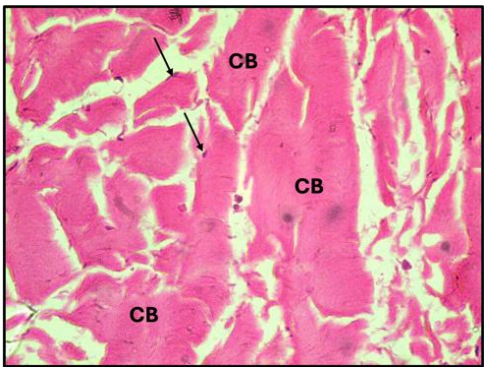

Figure 3: Transverse section of ligamentum teres femoris showing collagen bundles with fibres running in different directions. The fibroblasts nuclei (arrows) are situated close to the collagen bundles. CB: collagen bundles. Haematoxylin and eosin (40X).

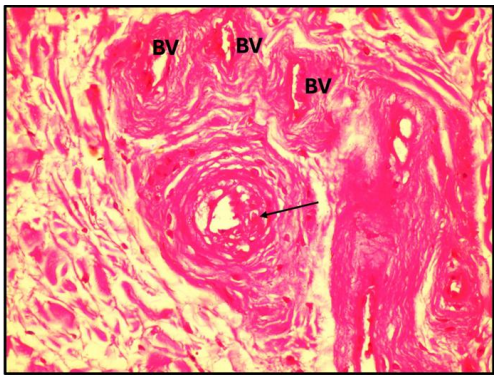

Figure 4: Transverse section of ligamentum teres femoris showing nerve bundles (arrow) in the sub-synovial tissue. BV: blood vessels. Haematoxylin and eosin (40X).

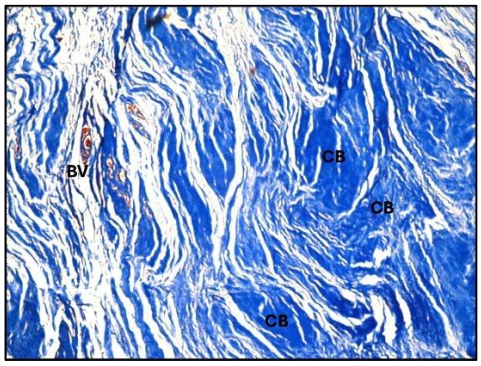

Figure 5: Transverse section of ligamentum teres femoris showing collagen bundles with fibres running in different directions. The blood vessels are also present in the loose connective tissue between the bundles. BV: blood vessels; CB: collagen bundles. Masson's Trichrome (40X).

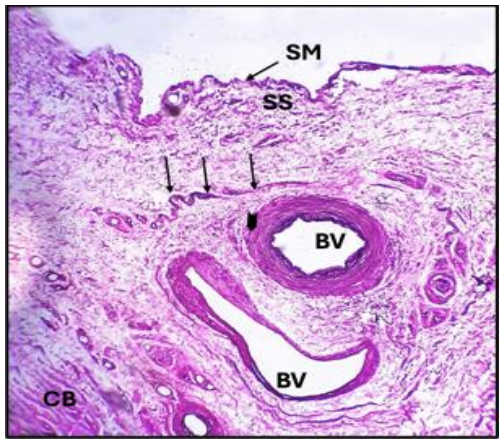

Figure 6: Transverse section of acetabular end of ligamentum teres femoris showing large blood vessels in the loose connective tissue below the synovial membrane. Many small and few medium sized vessels, and elastic fibres (arrows) are also seen in the connective tissue. SM: synovial membrane; SS: sub-synovial tissue; CB: collagen bundles; BV: blood vessels. Verhoeff van Gieson (4X).

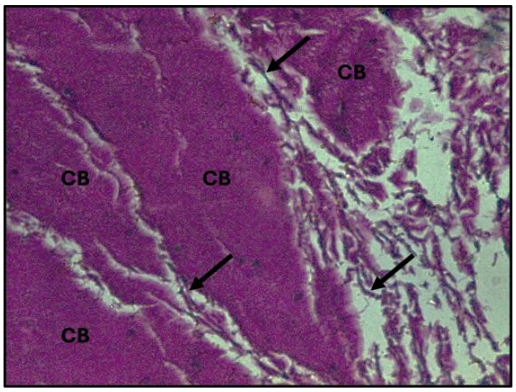

Figure 7: Transverse section of ligamentum teres femoris showing elastic fibres (arrows) in the loose connective tissue near the collagen bundles. CB: collagen bundles. Verhoeff Van Gieson (40X).

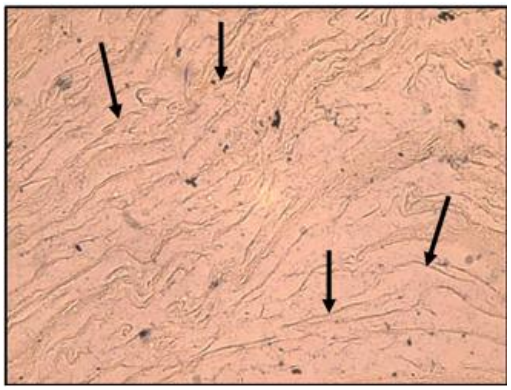

Figure 8: Transverse section of ligamentum teres femoris showing nerve fibres (arrows) scattered in the sub-synovial connective tissue. Palmgren's silver stain (40X).

The ligament was kept for two days in 10% neutral buffered formalin for fixation. Three segments of five mm thickness were taken from proximal (acetabular) and distal (femoral) ends, and middle of the LTF. The tissue samples were washed with normal saline after grossing and were transferred to the tissue cassettes for tissue processing. From each segment, the transverse tissue sections of 5 µm thickness were taken using the rotator microtome. The consecutive slides were stained with Haematoxylin and Eosin (H&E), Masson's trichome, Verhoeff's Van Gieson and Palmgren's silver stains. The major histological features like synovial membrane, blood vessels, connective tissue, and collagen bundles of three parts of ligament were observed in H&E. The special stains of Masson's trichrome and Verhoeff's Van Gieson were done to specifically note the arrangement of connective tissue components like collagen and elastic fibres, respectively in LTF. Palmgren silver was used to highlight presence of neural components like free nerve endings in the ligament. For silver stain, 15 µm thick sections were used. The slides were examined using the bright field compound microscope (Olympus CX41, Tokyo, Japan) under 4X, 10X and 40X. The general features were observed in H&E, collagen bundles in Masson's Trichrome, elastic fibres in Verhoeff's Van Gieson and nerve fibres in Palmgren silver-stained sections. The pictures taken with a digital camera fitted into microscope were uploaded into the Image J software (version 2.0) for measurements.

The morphometric parameters for blood vessels, collagen bundles and sub-synovial tissue were examined per section in H&E. It included number, diameter, and total luminal surface area of blood vessels, and number, and thickness of collagen bundles, and thickness of sub-synovial tissue. For histomorphometry, three serial sections from the two ends and middle of ligament were selected, such that total nine sections were examined for each specimen. All parameters were measured thrice by a single observer and the mean of three readings was taken as final.

All the data were analysed using IBM_ PASW STATISTICS version 20.0 (SPSS software version 20.0). After checking for normal distribution, the statistical difference between the data from the left and right sides was analysed by paired t-test. The statistical analysis was carried out with 5% level of significance and p-value < 0.05 was considered as statistically significant.

3. Results

An outer thin synovial layer was a single row of cells with round to oval nuclei all around the LTF (**Figure 1**). The sub-synovial tissue contained loose connective tissue, adipose tissue, scattered fibroblasts, numerous blood vessels, and nerve bundles. The collagen bundles were seen, below the sub-synovial tissue, in the substance of the ligament. The connective tissue of synovial folds, at the proximal and middle regions, was continuous with the sub-synovial connective tissue (**Figure 2**). It consisted of mainly small blood vessels, fibroblasts and adipocytes. The small arteries were consistently surrounded by adipose tissue and the medium to large vessels were noted near collagen bundles in the substance of ligament. The elastic fibres were distributed along the loose connective tissues in sub-synovial tissue. The multiple extensions from the loose connective tissue divided the substance of the LTF into multiple bundles containing collagen fibres (**Figure 4, Figure 8**). The collagen bundles were dense and compactly arranged surrounded by loose connective tissue, bundles of different sizes were arranged in parallel and oblique patterns. The longitudinally cut thin fibre bundles were wavy in appearance. The fibroblasts were dispersed between the collagen bundles. The nerve fibres were thin, short and scattered mainly in the sub-synovial area in all three parts of the ligament (**Figure 5**, **Figure 6**). In the substance of ligament, the elastic fibres were wavy and loosely arranged around collagen bundles, and were well-defined around large blood vessels (**Figure 7**).

At the proximal end, the collagen fibre bundles were mainly concentrated on the posterior aspect. The adipose tissue was seen in sub-synovial tissue and in the substance of the ligament sandwiched between the collagen fibre bundles. Multiple synovial folds were seen at the anterior edge of the ligament. These folds were filled with adipocytes, blood vessels along with loose connective tissue. Multiple small vessels were seen along the antero-inferior edge of the ligament and large sized vessels in central part surrounded by adipocytes.

In middle part, thick bundles of collagen fibres were seen in central and posterior part of the ligament. The anterior part of the ligament was filled with multiple small blood vessels surrounded by adipocytes and loose connective tissue. The small to medium sized vessels were seen at the inferior edge. In distal part, large collagen bundles were seen throughout the substance of the ligament. Small to medium sized vessels were seen along the inferior and posterior margins.

The number of blood vessels was significantly more in middle part of the LTF, whereas the number of blood vessels were fewer in distal part (**Table 1** and **Table 2**). The difference in number (p=0.922), diameter, luminal area of blood vessels between right and left sides was not statistically significant (**Table 3**). The average diameter of blood vessels was significantly more in distal part of LTF, and lower in middle part of the LTF when compared to other parts. Blood vessel luminal surface area was greater in the proximal part of LTF, and was lower in distal part.

Average number of collagen bundles was more in the proximal part of LTF, and was reduced in distal part (**Table 4** and **Table 5**). The difference in average number of collagen bundles between right and left sides was not statistically significant (p=0.414). Thickness of collagen bundles was more in middle part of LTF, and was reduced in distal part. Mean thickness of sub-synovial tissue was more in middle part of LTF, and was reduced in distal part when compared to proximal and middle parts (**Table 5**).

Table 1: The number, diameter and luminal surface area of blood vessels.

Part of ligament	Number (Mean ± SD)	*p value	Diameter (Mean ± SD) µm	**p value	Luminal surface area (Mean ± SD) µm2	**p value
Proximal	23 ± 6	< 0.001	51.2 ± 12.1	0.004	11029 ± 1580	< 0.001
Middle	27 ± 7		47.1 ± 6.1		8452 ± 1031	
Distal	20 ± 5		53.2 ± 6.5		5020 ± 1161	

*Kruskal-Wallis test; ** One-Way ANOVA test; SD- Standard deviation.

Table 2: Difference in the number, diameter and luminal surface area of blood vessels between three parts of ligament.

Variable	Parts of LTF		Mean difference	p -value
Number*	Proximal	Middle	- 4	0.009
		Distal	3	0.007
	Middle	Distal	7	0.000
Diameter ** (µm)	Proximal	Middle	4.1	0.077
		Distal	-2	0.847

	Middle	Distal	-6.1	0.003
Luminal surface area** (µm2)	Proximal	Middle	2577	<0.001
		Distal	6009	<0.001
	Middle	Distal	3432	<0.001

* Mann-Whitney test; ** Bonferroni post-hoc test.

Table 3: Morphometric measurements of ligament.

Parameter	Side	Minimum	Maximum	Mean	SD	p-value
Diameter of blood vessels (µm)	Right	31.95	75.73	51.08	8.6	
	Left	28.79	78.86	49.0	8.9	
	Total	28.79	78.86	50.5	8.9	0.478
Total luminal surface area of blood vessels (µm2)	Right	3381.97	14106.26	8083.9	2736.0	
	Left	3141.29	14390.16	8250.8	2838.4	
	Total	3141.29	14390.16	8167.4	2777.8	0.737
Thickness of collagen bundles (µm)	Right	77.24	183.85	112.0	22.6	
	Left	78.94	171.08	114.9	26.0	
	Total	77.24	193.12	113.4	24.3	0.498
Thickness of sub-synovial tissue (µm)	Right	158.5	274.68	208.0	31.1	
	Left	159.42	283.52	204.6	29.9	
	Total	154.16	283.52	206.3	30.4	0.536

SD- Standard deviation.

Table 4: Number and thickness of collagen bundles, and thickness of subsynovial tissue.

Part of ligament	Number (Mean ± SD)	*p value	Thickness (Mean ± SD) µm	**p-value	Subsynovial tissue thickness (Mean ± SD) µm	*p value
Proximal	15 ± 5	< 0.001	111.4 ± 25.6	<0.001	212 ± 25.6	< 0.001
Middle	14 ± 6		130.4 ± 21.6		229.2 ± 24.6	
Distal	9 ± 4		98 ± 12.6		177.7 ± 12.6	

*One-way ANOVA test; **Kruskal-Wallis test; SD – standard deviation.

Table 5: Difference in the number and thickness of collagen bundles, and thickness of subsynovial tissue between three parts of ligament.

Variable	Parts of LTF		Mean difference	p -value
Number*	Proximal	Middle	1	1.000
		Distal	6	0.001
	Middle	Distal	5	0.001
Thickness** (µm)	Proximal	Middle	-19	0.000
		Distal	13.4	0.016
	Middle	Distal	32.4	0.000
Subsynovial tissue thickness* (µm)	Proximal	Middle	-17.2	0.001
		Distal	34.3	0.000
	Middle	Distal	51.5	0.000

*Bonferroni Post-hoc test; ** Mann-Whitney test.

4. Discussion

In present study, the LTF of the hip was composed of a single layer of investing synovium, with an underlying connective tissue framework. The sections of synovial folds were seen more at the proximal and middle region; connective tissue of these folds was continuous with sub-synovial connective tissue. This finding agrees with microarchitecture study of LTF by Perumal et al.[7] Similarly, the arrangement of connective tissue encircling the entire LTF and sending extensions into the substance of the ligament was noted by Dehao et al. and Perumal et al.[7,14] The ligament proper below the synovial layer is composed of connective tissue and thick collagen bundles, this being comparable to the findings from Cerezal et al. and Perumal et al.[7,15]

Ippolito et al. mentioned the elastic fibres and small blood vessels between the collagen bundles, and Kirci et al. noted the presence of blood vessels in elderly cadavers below the synovial membrane but not in the central part.[16,1716,17] However, the blood vessels were visible in sub-synovial area as well as substance of ligament in LTF even though the majority of cadavers in the present study were in age range 65-75 years. Shinohara et al. reported that more collagen

bundles were present near ligament attachment site and the presence of type II collagen and fibrocartilage cells in the ligament attachment site was responsible for maintaining mechanical load and stresses produced by the LTF.[6] The fibroblasts were dispersed over the substance of LTF and more between collagen bundles, analogous to the findings from studies by Ippolito et al. and Cerezal et al.[15,17] Byrd and Jones, identified major blood vessels within substance of ligament.[17] Chandler and Kreuscher showed major blood vessels in both synovial layer and sub-synovial connective tissue.[19] Likewise in the present study, sub-synovial tissue of LTF was rich in blood vessels and the large blood vessels were seen in the substance of ligament especially at the proximal end.

Weathersby described a foveal artery from the obturator artery in most and from the medial circumflex femoral artery in a few cases. Separate foveolar branches appeared from those vessels in 6.7%, and remaining 23.9% were an anastomosis formed between the obturator and medial circumflex arteries which gave origin to the foveolar artery.[9] In children, the epiphysis is more dependent upon the retinacular vessels, and in adults the united epiphysis predominantly receives nutrient from foveolar vessels. This explains the greater frequency of avascular necrosis in children when compared to adults.[10] Treuta characterized the artery of LTF as age dependant. From birth up to 5 years, the vessels of LTF did not contribute to femoral head perfusion and after 7 years, it was frequently present.[5] Among 114 specimens studied by Chandler and Kreuscher, the LTF was relatively avascular in four, had large number of small vessels in eight, partial or complete sclerosis of the arteries in 16, and the arteries with mean diameter of 0.2 to 1.5 mm were seen in 86 specimens.[19] Chung found that the artery within the LTF did not reach the femoral head in 63% of specimens, and it provided one or two deep vessels to the centre of femoral head in 31%.[20] Scaglietti and Calandriello demonstrated lack of pathway of blood in the LTF in the direction of femoral epiphysis in CDH.[21] However, in Legg-Calve Perthes disease, the LTF has been suggested as a route of revascularization.[10,22]

Understanding the distribution of blood vessels over the LTF would give an appropriate knowledge regarding the healing process following LTF reconstruction surgeries. The distal part of the LTF was found to be the least vascular as it had fewer number and lesser luminal area of blood vessels compared with the proximal and middle parts in the present study. Perumal et al. reported that the number and the luminal surface area of blood vessels were lower in middle part of the LTF, and it was found to be a less vascular part. They also noted that the distal part of LTF had more vessels with small lumen compared with proximal and middle part of LTF.[16] The present study, however, demonstrated that the middle part of the LTF had more vasculature compared with proximal and distal part of LTF and difference was statistically significant. In a study by Dehao et al. the diameter of blood vessels in LTF ranged from 30-400 μm,[14] compared to 28.8-78.9 μm in the current study (**Table 1**).

The capacity of LTF to bear mechanical load is comparable to anterior cruciate ligament.[23] When subjected to excessive stress, the two ends of LTF are more susceptible to get torn than the middle part.[23,24] Among the two ends, foveal attachment is more likely to get damaged during strain in an adducted joint.[25] This correlates with the findings in the present study that the core of middle part of ligament had compactly arranged large diameter collagen bundles in contrast to the two ends. The number and thickness of collagen bundles was less in distal part compared to proximal and middle parts of LTF. The presence of collagen fibres in LTF is indicative of its biomechanical significance.[14] The number of collagen and elastic fibres is increased in conditions like congenital dislocation due to excessive mechanical burden.[26] In the pathological conditions such as developmental dysplasia of hip and dislocation, the collagen fibres increase in thickness.[27,28] It is known that LTF gets weakened with age and is more susceptible to injury in elderly age groups.[25] The average age of cadavers in the present study was 70 years and as ligament strength decreases with age, it has possibly affected the collagen content of ligament observed in this study. LTF contains large and small type I collagen fibres in its substance. The ligament is composed of primarily type I, III collagen and fibrocartilage cells present in the transitional zone described as enthesis. In case of torn or degenerated LTF, the enthesis loses its strength leading to a decreased ability to resist the mechanical forces.[29] Ippolito et al. reported the presence of thin elastic fibres evident between the collagen bundles, arranged along the long axis of the ligament which reflected the mechanical stresses and loading borne by the ligament. In CDH, the elastic fibres were thicker and more numerous than that in the normal hip, and fibrocartilaginous metaplasia was secondary to the mechanical stresses caused by dislocation of hip.[26]

The articular branch of obturator nerve innervates the LTF.[9] Perumal et al. described the presence of mechanoreceptors and free nerve endings (FNE) embedded along the middle third of the ligament indicating proprioceptive and nociceptive roles of the ligament.[7] Leunig et al. determined that the density of FNE in the LTF was higher than that reported in anterior cruciate ligament and iliotibial tract.[30\] Sarban et al. observed mechanoreceptors in LTF but only free nerve endings in one-third of ligament specimens.[27] Haversath et al.[31] described nerve fibres limited to the central part of LTF, whereas Perumal et al.[7] reported nerve bundles in sub-synovial tissue and fine fibres in the main substance of ligament. However, no sensory nerve fibres in LTF were noted by some authors.[32] In the present study, nerve fibres were observed in the sub-synovial tissue in all the three parts of LTF. Certain pathological conditions like arthrosis and developmental dysplasia of hip affect the presence of nerve tissue in LTF. The mechanoreceptors are

absent in developmental dysplasia, and number of FNE decreases in cases of hips arthrosis.[28,33]

LTF plays a main role in hip stabilization in flexion, adduction and external rotation, and is typically lax in hip abduction. LTF also plays a role in hip stability by preventing excessive movement of head of femur and hence prevents dislocation by keeping femur firmly in acetabular socket.[34] LTF injuries are typically associated with hip dislocation. Partial or complete tears may occur in the flexion-adduction stress in a sudden twisting injury.[1] Declamp et al. proposed the hip hyperabduction as a cause of LTF tears.[35] The patients with damaged or degenerated LTF commonly present with persistent hip pain, hip joint stiffness and restricted movements.[36] Many authors report improved patient outcome especially in cases with hip instability and chronic pain due to reconstructive procedures of LTF.[36] The conservation of normal anatomic architecture of ligament is essential to achieve the optimal functional status.

The present study focused on histological morphology of LTF for the better understanding of its structure and function. Moreover, this knowledge is likely to advance the understanding of pathological basis of disorders or injuries affecting LTF. It also supports the existing literature which cements the role of LTF in hip stability further encouraging reconstructive interventions in traumatic injuries. The awareness on internal arrangement of blood vessels and collagen bundles in different parts of ligament is likely to guide surgeons in restoration of its structure.

It had limitations, however, as it involved the cadaveric specimens, and the blood vessels might be shrunken due to fixation hampering the visibility and morphometric measurements of small blood vessels. Also, transversely running vessels could not be included as the study involved only the cross sections. Since majority of ligaments were from sample above 65 years, age-related changes like decrease in collagen content and reduced vascularity may restrict generality of the study findings. The analysis of larger sample from fresh specimens may be the focus of future studies.

5. Conclusion

The findings support a potential nutritive role of the ligamental vessels to fovea capitis of the adult head of femur. The middle part is more vascularized than the proximal and distal parts. The blood vessels run in the ligamentum teres femoris both in subs-synovial connective tissue and in the substance of the ligament. Nerve fibres identified in the sub-synovial area of the ligament also support its nociceptive role. In addition to these findings, presence of collagen bundles and elastic fibres suggests that ligamentum teres femoris mechanically contribute to stability of hip joint.

6. Source of Funding

None.

7. Conflict of Interest

None.

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