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Original Article

Subsartorial Compartments and Membranes in the Adductor Canal: Morphological, Histological and Immunohistochemical Study

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ABSTRACT

Background: The precise description of the adductor canal and its containing membranes has become an issue of great surgical and clinical importance. Neurovascular entrapment within the adductor canal may simulate many clinical conditions for cases presented with medial knee or leg pain and ischemic manifestations of the leg.

Aim of the work: The The aim of the present work was to spotlight on the subsartorial fascial compartments in the adductor canal and to elucidate the morphological, histological and immunohistochemical features of the containing membranes.

Materials and Methods: Forty thigh specimens, pertaining to 15 embalmed and 5 fresh human adult cadavers (15 males, 5 females) were dissected in pursuit of this aim. Trichrome staining and immunohistochemical staining using skeletal muscle actin mouse monoclonal antibody were used to elucidate the structure of these membranes.

Results: Thick pentagonal vastoadductor membrane (VAM), distally, and thin triangular vastofemoral membrane (VFM), proximally, roofing two corresponding fascial tunnels and the subsartorial space superficial to them were observed. The mean length of VAM and VFM were 7.9 and 7.8 cm respectively. The VAM stretched between the vastus medialis (VM) and the adductor magnus (AM) and longus muscles and overlaid the saphenous nerve, its subsartorial and lower medial femoral cutaneous branches, femoral vessels, one to three arterial pedicles for the sartorius, one pedicle for the vastus medialis muscle and descending genicular vessels. The VAM originated from the tendinous fibres of the adductor magnus tendon and constantly spread anterolaterally. The arterial pedicles to sartorius muscle, the lower medial femoral cutaneous and the subsartorial branches of the saphenous nerve constantly pierced the VAM. An arterial pedicle to the vastus medialis muscle and perforating veins between the superficial veins and the femoral vein proved to pierce the VAM in 8/40 specimens. The VFM stretched between the femoral artery and the VM muscle and overlaid the femoral vessels, the saphenous nerve, nerve to the VM and an arterial pedicle for VM. It was pierced constantly by two arterial pedicles to the sartorius muscle and occasionally (20/40 specimens) by a communicating nerve between the saphenous and the medial femoral cutaneous nerves. Trichrome staining of the VAM revealed that it consisted of three superimposed layers of collagen bundles, appeared in oblique and longitudinal directions and having the same structural configuration and continuous with the tendinous fascia overlying the AM muscle. The VFM was formed of multiple thin transversely-cut collagen bundles. Immunohistochemical study proved the absence of the skeletal muscle fibres in both membranes as proved by negative immunoreaction.

Conclusions: The morphological and histological features of these subsartorial membranes may help the physicians to improve the management techniques of various clinical cases.

Key Words: Adductor canal, Vastoadductor membrane, Subsartorial compartment, medial intermuscular septum, collagen fibres, histological study, morphological study

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INTRODUCTION

The subsartorial or adductor canal was first described by John Hunter in 1786 and has been classically described as being conical or pyramid-shaped and contains the femoral vessels and saphenous nerve along with a varying amount of fibrous tissue *(De Oliveira et al., 2009)*. Further report has suggested a proximal wide base and narrow distal apex at the adductor hiatus; the narrowest point *(De Souza et al., 1978)*.

From anatomical perspectives the adductor canal, as described in anatomy textbooks, is an aponeurotic tunnel in the middle third of the thigh, extending from the apex of the femoral triangle, the point at which sartorius crosses adductor longus, to the adductor magnus hiatus *(Agur and Dalley, 2013; Netter et al., 2013; Standring, 2016)*. The lateral (vastus medialis) and posterior (adductor longus and magnus) walls of the canal were reported to be covered by a thick fascia *(Standring, 2016)*.

The vastoadductor membrane (VAM) is a term first described by Callander (1939) to be a deep fascia layer that roofs the adductor canal. He named it as the fascia vasto-adductoria. Weinstabl et al. (1989) have included the vastoadductor "lamina" as an attachment site for the vastus medialis muscle.

The clinical importance of the VAM is ascribed to being a boundary for subsartorial fibromuscular tunnel and stretched over the saphenous nerve and femoral vessels. Neurovascular compression within this tunnel should be remembered while diagnosing cases presented with medial knee or leg pain and ischemic manifestations of the leg.

It is noteworthy that saphenous nerve neuralgia mimics a number of clinical conditions including lumbar radiculopathy *(Saal et al., 1988; Ahadi et al., 2010)*, patellofemoral disorders *(Saal et al., 1988)*, suprapatellar plica *(Saal et al., 1988)*, medial meniscus tear *(Espejo et al., 2007)*, tibial stress fracture *(Peck et al., 2010)*, pes anserine tendonopathy, bursitis *(Ahadi et al., 2010; Peck et al., 2010)*, osteochondritis dissecans (Saal et al., 1988), nonspecific synovitis *(Saal et al., 1988)* and reflex sympathetic dystrophy *(Katz and Hungerford, 1987; Poehling et al., 1988; Kaplan, 1989; Finsterbush et al., 1991; Ahadi et al., 2010)*.

Saphenous nerve neuralgia may be attributed to entrapment, trauma or surgical intervention. The exit of the saphenous nerve in relation to the VAM is reported to be a site for entrapment *(Verma et al., 2013)*. The main complaints associated with saphenous nerve entrapment include pain along the saphenous nerve distribution following prolonged walking or standing or quadriceps exercise *(Sunderland, 1978)*. Stair climbing may aggravate the symptoms *(McKechnie, 1995)*.

Claudication syndrome, associated with femoral artery compression within the adductor canal, typically presented in young males during exercise suggesting that adjacent muscles hypertrophy plays an important role in the pathophysiological mechanism *(Pratt, 1971)*.

Despite the clinical relevance of the subsartorial compartments and membranes, few studies describing their anatomical features exist. The aim of the present work was to clarify the subsartorial fascial compartments in the adductor canal, the morphological features and the relationships of the containing subsartorial membranes. Histological and immunohistochemical studies were done to elucidate their structure. This may help the orthopaedic surgeons, radiologists, anaesthesiologists in addition to pain and sports medicine physicians to improve the management techniques of various clinical cases.

MATERIALS AND METHODS

Cadaveric specimens

A total of 40 thigh specimens, pertaining to 15 embalmed and 5 fresh human adult cadavers, aged 30 through 60 years (15 males, 5 females), obtained from the Anatomy & Embryology Department, Faculty of medicine, Alexandria University were subjected to this study. None of the specimens had signs of pathological lesions, previous trauma or surgery. The fresh cadavers were used within 12 hours after death and the embalmed cadavers were fixed in 4% formaldehyde for at least 6 months post-mortem. All cadavers were included in the present study after the approval of the institutional ethics committee.

Cadaveric dissection

The skin incision was made along the lower two-thirds of a line joining the anterior superior iliac spine and the adductor tubercle. After elevation and lateral retraction of the skin flap, identification of the sartorius muscle and incision of the deep fascia along its posterior border were done to expose the subsartorial compartments. The adductor canal was delineated from a point of crossing of the medial border of both the sartorius and adductor longus muscles proximally to the adductor hiatus distally.

Blunt dissection for the subsartorial compartments in the adductor canal with preservation of containing nerves and vessels was done. The subsartorial membranes were identified and their shape, borders, attachments, direction of fibres and structures piercing them were reported. Their length, width at the proximal and distal borders and their distance from the adductor tubercle and the upper border of the patella were measured. The widest width of the VAM was also measured. Their relation to the neighbouring nerves and vessels were reported and photographed.

Histological preparation

Tissue sampling and preparation for light microscopic examination:

From each of the five fresh and 15 embalmed cadavers a three cm long rectangular block was dissected at the widest part of the fascial tunnels deep to the subsartorial membranes. The specimens were fixed immediately in 10 % formol saline for 72 hours at 4°C. Paraffin blocks were prepared and 5 µm thick sections were stained using Masson's trichrome *(Bradbury and Rae, 1996)*. The collagen fibres appeared green in colour.

Immunohistochemistry (IHC)

Immunohistochemical staining for detection of the skeletal muscle was done, in the five fresh cadavers specimens, by using Actin, skeletal muscle Ab-2 (clone 5C5.F8.C7 or α-Sr-1) mouse monoclonal antibody (MAb). This MAb-immunoglobulin together with DAB chromogen (3'-Diamino benzidine tetrahydrochloride) and haematoxyline counterstaining gives brownish deposits at the sites of positive reaction *(Cintorino et al., 1989)*.

Statistical Analysis

All distances were measured by metallic ruler. Each measurement was taken twice at different times; when the difference was less than 2 mm, the mean was calculated. When the difference was greater, a third reading was considered and the mean recorded. The mean and standard deviation were calculated. Statistical Package for the Social Sciences (SPSS) program version 11.5 was used for data entry and analysis with significance set at $P<0.05$. To evaluate the statistical significant difference for these measurements among the specimens in two sex groups (30 males and 10 females) and two side groups (20 right and 20 left) Student's t- test was done.

RESULTS

Morphological results

The subsartorial compartments:

In the subsartorial canal, two membranes namely the vastoadductor and the vastofemoral, roofing two corresponding fascial tunnels could be identified (Fig. 1). Superficial to them and deep to the deep layer of fascia lata split enveloping the sartorius muscle there was another space, namely subsartorial space, which contained the neurovascular structures after piercing the former two membranes, on their way to their final destination (Figs. 1,2).

The vastoadductor membrane:

Morphology and attachment:

The vastoadductor membrane (VAM) proved to be a constant thick pentagonal aponeurotic fibrous sheet having five borders; proximal, distal, anterolateral and two posteromedial; long distal and short proximal (Fig. 3). It stretched between the medial margin of each of the vastus medialis (VM) anterolaterally, and the tendinous margin of the adductor magnus (AM) distal posteromedially (Fig. 3). The proximal posteromedial attachment laid on the adductor magnus and a small part of the adductor longus (AL) before its termination just medial to the femoral artery (Fig. 4).

The proximal border stretched between the vastus medialis and a point on the anterior surface of the adductor longus muscle just medial to the femoral artery. It was either continuous (Fig. 3), in 34/40 specimens (85%), or discontinuous

(Fig. 4), in 6/40 specimens (15%), with the distal border of another membrane, deep to sartorius muscle. This membrane could be named the vastofemoral membrane (VFM). The shorter distal border of the VAM stretched between the distal end of the tendinous margin of the adductor magnus and the vastus medialis muscle (Fig. 4).

The tendinous fibres of the adductor magnus tendon constantly spread anterolaterally on its surface to form the VAM (Fig. 3). In 30/40 specimens (75 %) the proximal, middle and distal fibres ran upwards, transversely and downwards respectively (Figs. 3,5). In 6/40 specimens (15%) and 4/40 specimens (10%) the fibres coursed upwards (Fig. 6) and downwards (Fig. 7) respectively. Thicker brownish striated appearance of the distal two thirds of the VAM was observed in 6/40 specimens (15%; Fig. 6).

The vastoadductor tunnel:

The space deep to VAM could be named “vastoadductor tunnel”. It had wide proximal and narrow distal ends. It was bounded by the vastus medialis anterolaterally, VAM anteromedially and the adductor magnus and small contribution for the adductor longus posteriorly (Fig. 8). The vastoadductor tunnel contained fat, the femoral and the descending genicular vessels and the saphenous nerve (Figs. 6,8). Additionally, it contained the origin of the arterial pedicles from the femoral artery to the vastus medialis and the sartorius muscles as well as the terminations of veins drained into the femoral vein. The lower medial femoral cutaneous branch and the subsartorial branches of the saphenous nerve proved to be contents in the tunnel before piercing the VAM (Fig. 7). Throughout the tunnel the femoral vein was inferolateral to the artery (Fig. 8). The descending genicular vessels and saphenous nerve constantly coursed downwards anterior to the femoral artery and exit the tunnel through its distal aperture deep to the distal border of the VAM (Figs. 4,6).

Structures piercing the VAM:

From one to three vascular pedicles, arising from the femoral artery, to sartorius muscle (Figs. 1,4,7) and the lower medial femoral cutaneous branch of the saphenous nerve, on its way to the skin, constantly pierced the VAM (Figs. 1,9). The subsartorial branches of the saphenous nerve proved to pierce the VAM to anastomose with the anterior branch of the obturator nerve on the deep surface of the sartorius muscle in the subsartorial space to share in the subsartorial plexus of nerves (Fig. 7). An arterial pedicle to the vastus medialis muscle (Fig. 9) and perforating veins between the superficial veins and the femoral vein proved to pierce the VAM in 8/40 specimens (20%; Figs. 1,7,9).

One arterial pedicle and a muscular nerve branch, arising from the femoral artery and nerve respectively, constantly descended superficial to the VAM en route to the sartorius muscle (Figs. 3,4,6,9).

The vastofemoral membrane (VFM)

Morphology and attachment:

The vastofemoral membrane proved to be a constant subsartorial thin triangular fibrous sheet with proximal apex and distal base. It stretched between the femoral artery, posteromedially and the vastus medialis (VM) muscle, anterolaterally (Figs. 3,4).

The vasofemoral tunnel:

The space deep to it namely, the vastofemoral tunnel contained fat, the femoral vessels, the saphenous nerve, an arterial pedicle for VM and nerve to the VM (Figs. 10,11). The saphenous nerve and femoral vein were anterolateral and posterolateral to the artery respectively (Fig. 10) while the nerve to VM coursed lateral to the femoral artery (Fig. 11).

Structures piercing the VFM:

It was constantly pierced by two arterial pedicles arising from the femoral artery to the sartorius muscle (Fig. 4) and occasionally by a communicating nerve between the saphenous and the medial femoral cutaneous nerves (20/40 specimens, 50%; Figs. 4,11).

Statistical analysis

Apart from the greater measurements in male, no statistically significant differences was found as regards sex and side of the specimens (P= 0.099). Tables 1 and 2 summarize the descriptive statistics for the outcome variables of the VAM and VFM respectively. The point of convergence between the distal and proximal posteromedial borders coincided with the widest width of the VAM.

Histological results

The vastoadductor membrane:

Trichrome stain: The VAM was observed to be formed of three superimposed dense layers of green-stained collagen fibres (Fig. 12). Their direction was either longitudinal or oblique. Such collagen bundles appeared to have similar structural configuration and were continuous with those covering the adductor magnus (AM) muscle (Fig. 13). Thick bundles of the fascia covering the vastus medialis muscle were observed to be formed of closely packed, green-stained, oblique and longitudinal collagen fibres (Fig. 14). The collagen fibres of the AM aponeurosis, having the same direction of the muscle fibres, appeared as transversely sectioned bundles (Fig. 13).

Immunohistochemical staining of skeletal muscle actin:

The VAM revealed negative immunoreaction for the skeletal muscle actin, whereas the vastus medialis and the adductor magnus muscles showed positive reaction as reddish brown-stained, transversely sectioned, skeletal muscle fibres (Fig. 15).

The vastofemoral membrane:

Trichrome stain: The VFM was observed to be formed of multiple thin green-stained collagen bundles appeared transversely sectioned (Fig. 16). The vastus medialis fascia near the attachment of the VFM became thin and appeared as green-stained collagen bundles in transverse and oblique directions (Fig. 17).

Immunohistochemical staining of skeletal muscle actin:

The VFM revealed negative immunoreaction for the skeletal muscle actin, whereas the vastus medialis showed positive reaction of reddish brown-stained, transversely sectioned, skeletal muscle fibres (Fig. 18).

Table 1: Descriptive statistics for the outcome variables of the vastoadductor membrane among the dissected 20 cadavers (40 thighs).

Measurements		Range (cm)	Mean ± SD
Length		5.4 – 10	7.9 ± 1.698
Width	proximal border	1.27 – 2	1.6 ± 0.213
	Distal border	0.6 – 0.8	0.7 ± 0.057
	Widest width	1.6 – 2.5	2.1 ± 0.287
Distance from the adductor tubercle to	proximal border	15.8 – 17	16.4 ± 0.433
	Distal border	6.7 – 9	7.7 ± 0.695
	Point of widest width	13.6 – 15	13.9 ± 2.093
Distance from the upper border of the patella to	proximal border	14.5 – 16	15.3 ± 0.486
	Distal border	6 – 8	7 ± 0.55

Table 2: Descriptive statistics for the outcome variables of the vastofemoral membrane among the dissected 20 cadavers (40 thighs).

Measurements		Range (cm)	Mean ± SD
Length		7 – 8.5	7.8 ± 0.472
Width	proximal border	0	0
	Distal border	0.8 – 1.3	1 ± 0.164
Distance from the adductor tubercle to	proximal border	23.5 –29	26.2 ± 1.688
	Distal border	16.5 – 17.3	16.9 ± 0.245
Distance from the upper border of the patella to	proximal border	22 – 28	25.6 ± 1.531
	Distal border	15.5 – 16.5	16 ± 0.35

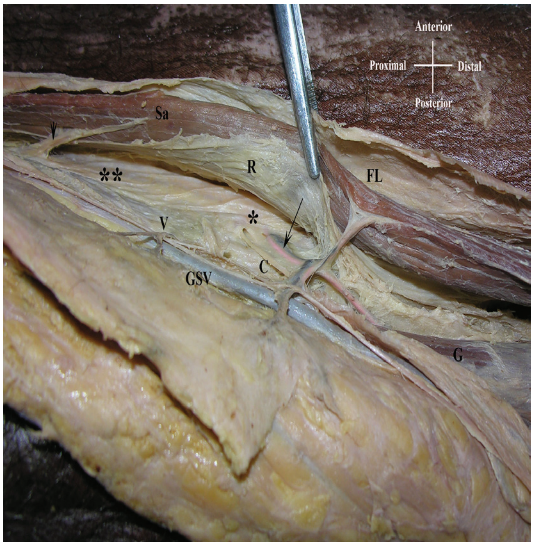

Fig. 1: Photograph of the left subsartorial compartment in preserved cadaver, medial view, showing the deep layer of fascia lata split (R) enveloping the sartorius muscle (Sa) that roofs the subsartorial space. The lower femoral cutaneous branch (C) of the saphenous nerve and a vascular pedicle (arrow) for the sartorius muscle are seen piercing the vastoadductor membrane (asterisk). Notice the presence of communicating vein (V) between the femoral vein and superficial veins. Arrow head, muscular branch for the sartorius muscle; double asterisks, vastoafemoral membrane; FL, fascia lata; G, gracilis; GSV, great saphenous vein.

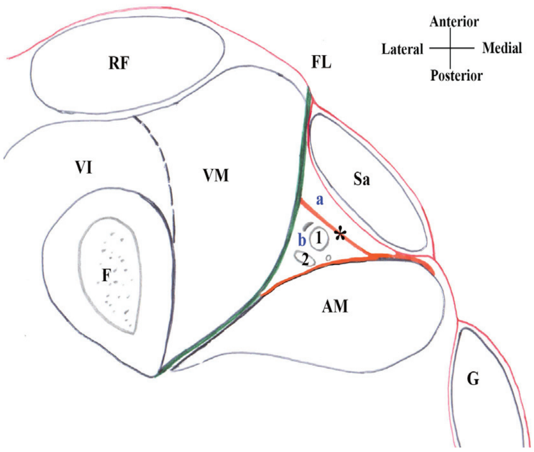

Fig. 2: Diagrammatic illustration for the transverse section of the left adductor canal, proximal view, showing the subsartorial space (a) and the vastoadductor tunnel (b) deep to the sartorius muscle (Sa). The green line points to the medial intermuscular septum extending from the linea aspera of femur (F) to the fascia lata (FL), red line, on the anterior border of sartorius muscle. The orange line points to the tendinous fibres on the surface of the adductor magnus (AM). The vastoadductor membrane (asterisk) originates from these tendinous fibres. The roof of the adductor canal is formed by the deep layer of FL split enveloping the sartorius muscle. Femoral artery (1) and vein (2) course deep to the vastoadductor membrane. VI, vastus intermedius; RF, rectus femoris; G, gracilis**.**

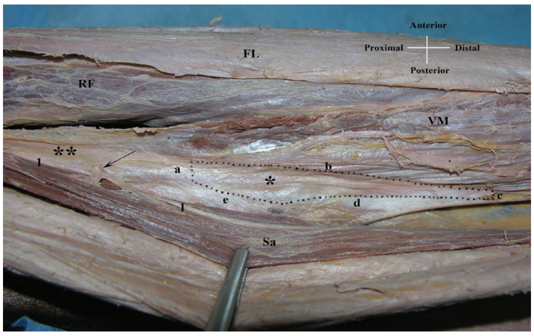

Fig. 3: Photograph of the left subsartorial compartment in preserved cadaver, medial view, showing the pentagonal shape and attachment (dotted line) of the five borders of the vastoadductor membrane (asterisk); proximal (a), anterolateral (b), distal (c) and two posteromedial; distal (d) and proximal (e). It is continuous proximally with the vastofemoral membrane (double asterisks). Notice the anterolateral fibres direction of the vastoadductor membrane; upwards, transverse and downwards in the upper, middle and lower parts respectively. The muscular nerve branch (1) and an arterial pedicle (arrow) for the sartorius muscle (Sa) descends superficial to vastofemoral and vastoadductor membranes. VM, vastus medialis; RF, rectus femoris; FL, fascia lata.

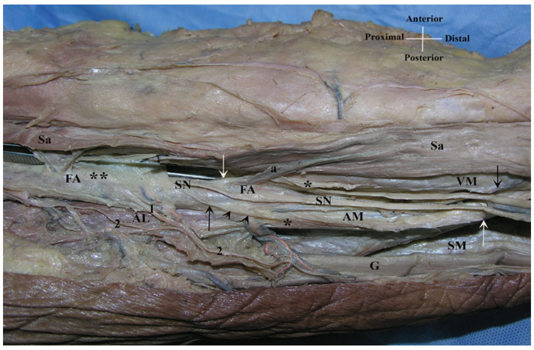

Fig. 4: Photograph of the left subsartorial compartment in preserved cadaver, medial view, the sartorius muscle (Sa) is retracted laterally to show the attachment of the vastoadductor membrane (asterisk) to the tendinous margin of the adductor magnus (AM) and the vastus medialis (VM). The posteromedial attachment spans proximally (arrowheads) across the adductor longus (AL) to reach the femoral artery (FA). Notice the proximal and distal limits (four arrows) of the vastoadductor membrane and the arterial pedicle (a) to the sartorius muscle. The saphenous nerve (SN) descended anterior to the FA in the vastoadductor tunnel. The vastofemoral membrane (double asterisks) is seen stretched between the FA and the VM and pierced by a communicating nerve branch (1) between the SN and the medial femoral cutaneous nerve (2). The forceps is passing through the vastofemoral tunnel. Notice the discontinuation between the vastoadductor and vastofemoral membranes. G, gracilis; SM, semimembranosus.

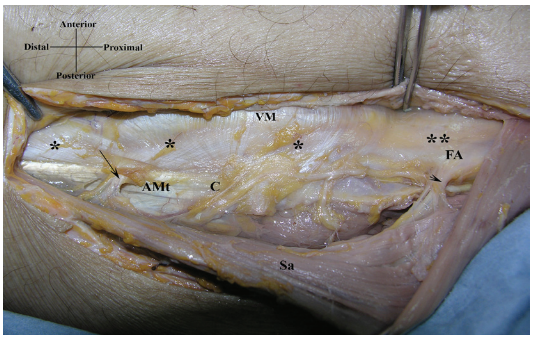

Fig. 5: Photograph of the right subsartorial compartment in fresh cadaver, medial view, showing the upwards, transverse and downwards anterolateral direction of the vastoadductor membrane (asterisk) that is pierced by the lower medial femoral cutaneous branch of the saphenous nerve (C). Two arterial pedicles (arrowhead) arising from the femoral artery (FA) to the sartorius muscle (Sa) and another one (arrow) are seen piercing the vastofemoral (double asterisks) and vastoadductor membranes respectively. AMt, adductor magnus tendon; VM, vastus medialis.

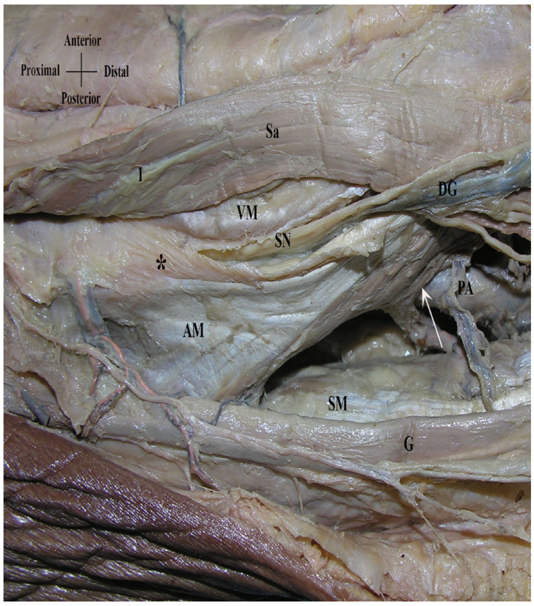

Fig. 6: Photograph of the left subsartorial compartment in preserved cadaver, medial view, the sartorius muscle (Sa) is retracted laterally to show the upwards and lateral direction of thick vastoadductor membrane (asterisk) and the exit of the saphenous nerve (SN) with the descending genicular (DG) vessels through the distal aperture of the vastoadductor tunnel. The distal part of the vastoadductor membrane has striated appearance and is brownish in colour. Notice the course of the muscular nerve branch (1) to the under- surface of the sartorius muscle and the exit of the popliteal artery (PA) from the adductor hiatus (arrow). G, gracilis; SM, semimembranosus; AM, adductor magnus; VM, vastus medialis.

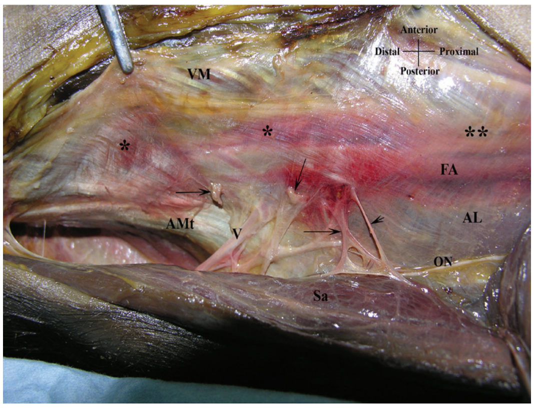

Fig. 7: Photograph of the right subsartorial compartment in fresh cadaver, medial view, showing the thick fascia on the vastus medialis (VM), stretched by two forceps. The sartorius muscle (Sa) is retracted medially to show the downwards anterolateral direction of the vastoadductor membrane (asterisk). It is pierced by three arterial pedicles (arrows) to the sartorius muscle and a communicating vein (V) between the femoral vein and the superficial veins. The subsartorial branch of the saphenous nerve (arrowhead) pierced the vastoadductor membrane to anastomose with anterior division of the obturator nerve (ON) on the deep surface of the sartorius muscle in the subsartorial space. The femoral artery (FA) could be seen deep to the thin vastofemoral membrane (double asterisks). AL, adductor longus; AMt, adductor magnus tendon.

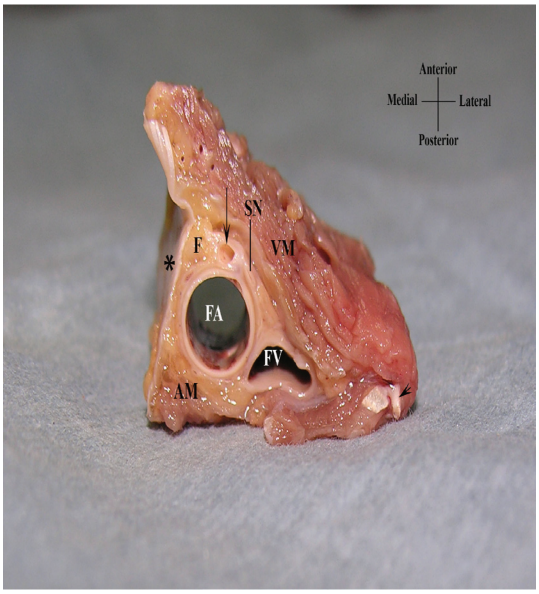

Fig. 8: Photograph of a rectangular block specimen for the right vastoadductor tunnel in fresh cadaver, proximal view, showing its boundaries and contents. asterisk, vastoadductor membrane; VM, vastus medialis; AM, adductor magnus; FA, femoral artery; FV, femoral vein; SN, saphenous nerve; F, fat; arrow, arterial pedicle for the VM; arrowhead, nerve to VM.

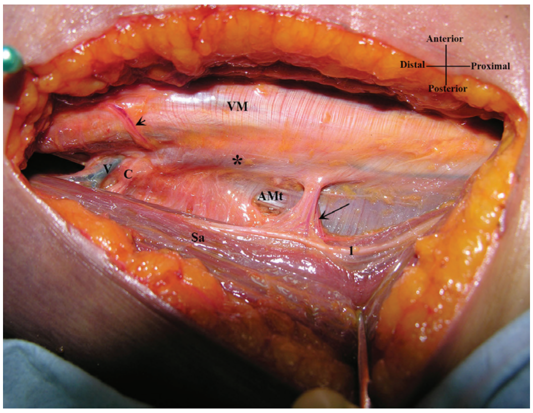

Fig. 9: Photograph of the right subsartorial compartment in fresh cadaver, medial view, showing the thick fascia on the vastus medialis (VM) receiving the attachment of the vastoadductor membrane (asterisk). An arterial pedicle (arrow) to the sartorius muscle (Sa) and other (arrow head) for the vastus medialis (VM), the lower femoral cutaneous branch (C) of the saphenous nerve and a communicating vein (V) between the femoral vein and superficial veins are seen piercing the vastoadductor membrane. Notice the course of the muscular nerve branch (1) to the sartorius muscle. AMt, adductor magnus tendon.

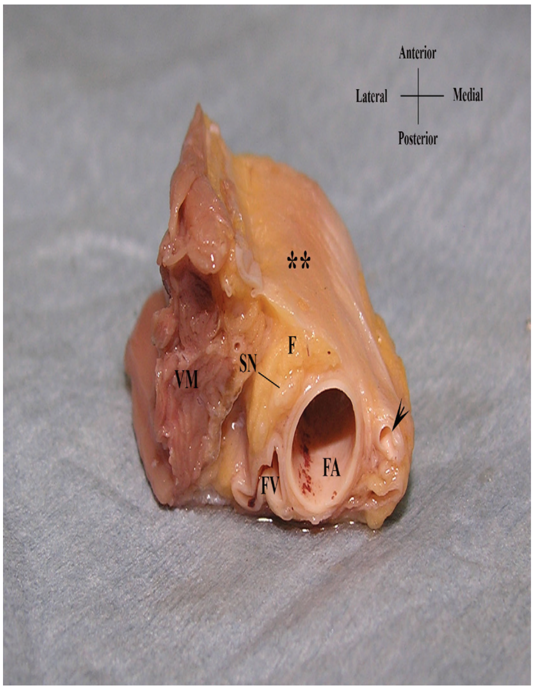

Fig. 10: Photograph of a rectangular block specimen for the right vastoafemoral tunnel in fresh cadaver, distal view, showing its boundaries and contents. Double asterisks, vastofemoral membrane; VM, vastus medialis; FA, femoral artery; FV, femoral vein; SN, saphenous nerve; arrowhead, arterial pedicle for the sartorius muscle; F, fat.

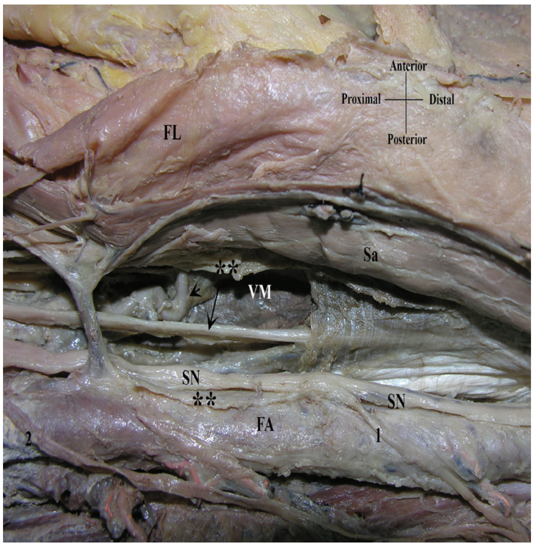

Fig. 11: Photograph of the left subsartorial compartment in preserved cadaver, medial view, the sartorius muscle (Sa) is retracted laterally and the vastofemoral membrane (double asterisks) is cut to show the contents of the vastofemoral tunnel; saphenous nerve (SN), nerve (arrow) and arterial pedicle (arrowhead) to vastus medialis (VM). A communicating nerve branch (1) between the SN and the medial femoral cutaneous nerve (2) is seen. FA, femoral artery; FL, fascia lata..

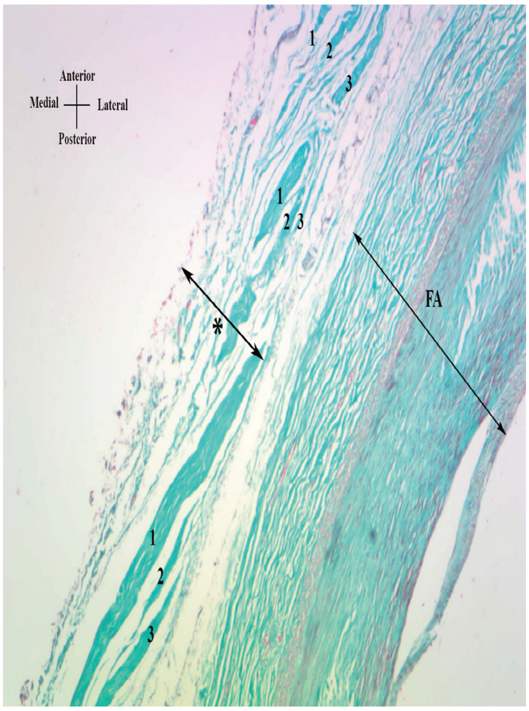

Fig. 12: Light photomicrograph of a transverse section of the left vastoadductor membrane (asterisk), distal view, showing three superimposed layers of green-stained longitudinal collagen bundles (1,2,3). FA, wall of the femoral artery (Masson's trichrome stain, X 40).

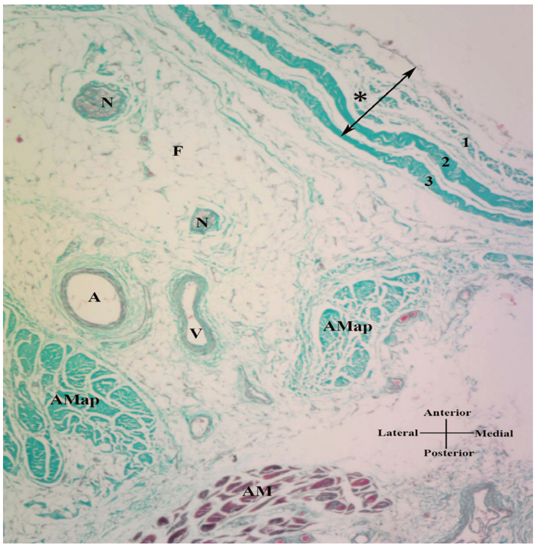

Fig. 13: Light photomicrograph of a transverse section of the left vastoadductor membrane (asterisk) near its attachment to the collagen bundles of the adductor magnus aponeurosis (AMap), proximal view, showing an outer oblique (1) and two inner longitudinal (2,3) layers of green-stained collagen bundles. The transverse direction of both green-stained collagen bundles of the AMap and the red-stained adductor magnus (AM) muscle fibres are also observed. Notice the presence of neurovascular branches; nerve (N), vein (V) and artery (A) in the adipose tissue (F) deep to the membrane.

(Masson's trichrome stain, X 40)

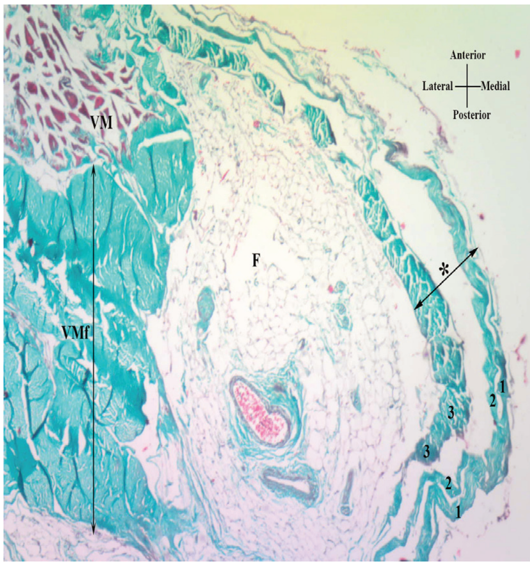

Fig. 14: Light photomicrograph of a transverse section of the left vastoadductor membrane (asterisk) at its attachment to the thick fascia (VMf) of the vastus medialis muscle (VM), proximal view, showing outer two longitudinal (1,2) and an inner oblique (3) layers of green-stained collagen bundles. The VMf is observed to be formed of thick layer of densely packed, green-stained, oblique and longitudinal collagen bundles.

(Masson's trichrome stain, X 40)

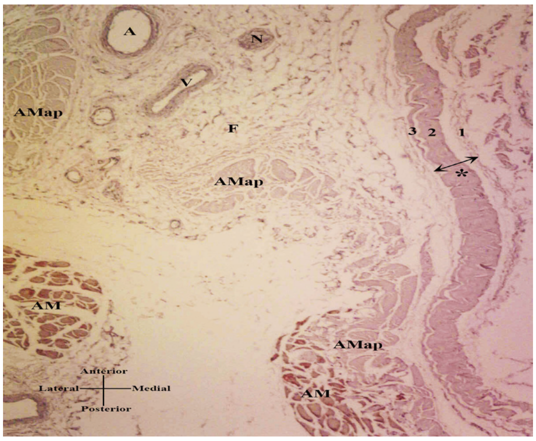

Fig. 15: Light photomicrograph of a transverse section of the left vastoadductor membrane (asterisk) at its attachment to the collagen bundles of the adductor magnus aponeurosis (AMap), proximal view, showing negative immunoreaction of the three superimposed layers of the collagen bundles (1,2,3). The adductor magnus (AM) muscle fibres express positive reddish brown immunoreaction. Notice the neurovascular branches; nerve (N), vein (V) and artery (A) in the adipose tissue (F) deep to the membrane.

(Skeletal muscle actin immunostain, X 40**).**

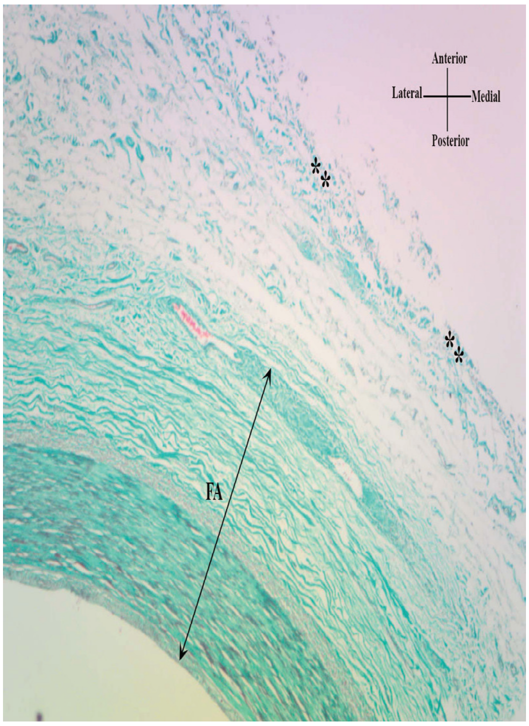

Fig. 16: Light photomicrograph of a transverse section of the right vastofemoral membrane (double asterisks), distal view, showing multiple green-stained thin bundles of transversely-cut collagen fibres. FA, wall of the femoral artery.

(Masson's trichrome stain, x 40).

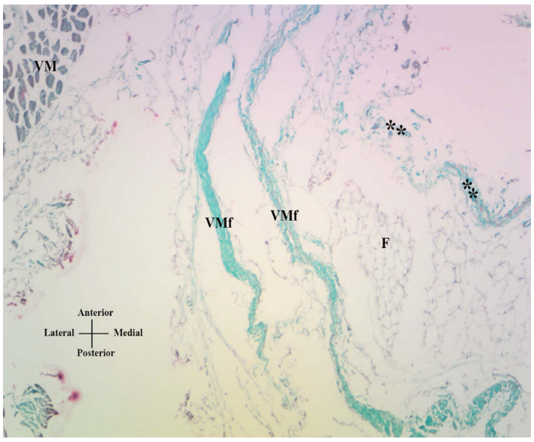

Fig. 17: Light photomicrograph of a transverse section of the right vastofemoral membrane (double asterisks) near its attachment to the vastus medialis muscle (VM), distal view, showing its multiple thin bundles of green-stained collagen fibres appeared in transverse direction. Notice the thin green-stained collagen bundles of the VM fascia (VMf) running in oblique and transverse directions. F, adipose tissue.

(Masson's trichrome stain, x 40).

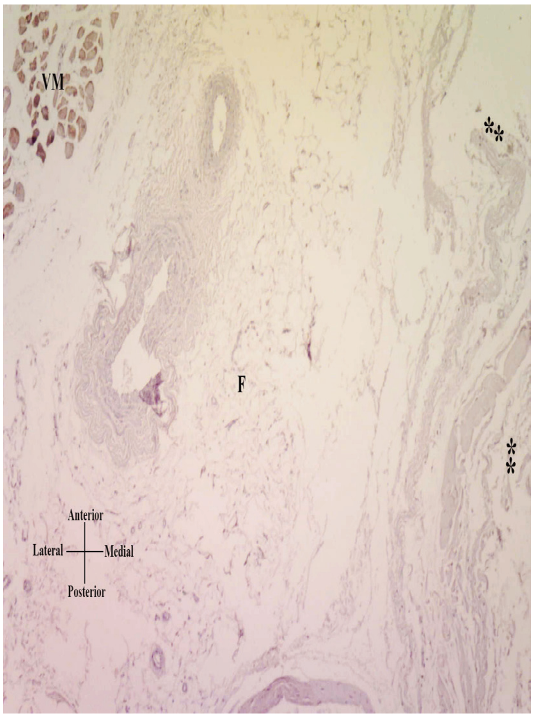

Fig. 18: Light photomicrograph of a transverse section of the right vastofemoral membrane (double asterisks) near its attachment to the vastus medialis muscle (VM), distal view, showing negative immunoreaction of the membrane. Positive immunoreaction of the VM muscle fibres, reddish brown-stained, is noticed. F, adipose tissue.

(Skeletal muscle actin immunostain, x 40).

DISCUSSION

The precise description of the anatomy of the subsartorial or adductor canal and its containing membranes and fascial compartments should pursue the remarkable diagnostic and therapeutic techniques for cases who presented with medial knee or leg pain and ischemic manifestation of the leg. The present study hypothesizes that the adductor canal included three fascial compartments. The first and second, namely vastoadductor and the vastofemoral tunnels, were roofed by two corresponding membranes. The subsartorial space, the third one, was superficial to both membranes and roofed by the deep layer of fascia lata split enveloping the sartorius muscle forming the roof of the adductor canal. The subsartorial space communicated with the first two tunnels in cases where the two membranes were discontinuous. This hypothesis may solve the debate around the precise descriptive relation of the VAM to the adductor canal.

It has been reported that the VAM formed the roof of the adductor canal *(Callander, 1939; Testut and Jacob, 1956; Hollinshead, 1958; De Souza et al., 1978, 1984; De Oliveira et al., 2009; Horn et al., 2009; Saranteas et al., 2011; Verma et al., 2013; Bendtsen et al., 2014; Andersen et al., 2015)*. Whereas the present study, in agreement with Woodburne (1978) and *Tubbs et al. (2007)* stated that the VAM forms a fascial compartment within the adductor canal. Nevertheless there is a consensus that the VAM overlies the saphenous nerve and the femoral vessels in the middle third of the thigh.

Bendtsen et al. (2014) described the subsartorial space inferior to the femoral triangle and superior to the VAM as being outside the adductor canal. *Andersen et al. (2015)* dissected the adductor canal in 15 embalmed cadavers and reported the subsartorial space to be superficial to the fascial roof of the adductor canal and named it as subsartorial fat compartment.

Knowing the definite morphology of the VAM has become an important issue for many clinicians. Being a potential cause for neurovascular compression and a structure confronted with in many surgical operations, it has gained a reasonable surgical importance for research study. *Checroun et al. (1996)* reported that during surgical medial approach to the femur the adductor canal hiatus was consistently located posterolaterally, hidden behind the VAM. Additionally the canalicular appearance of the proximal part of the vastoadductor canal may be mistaken for the adductor hiatus *(Checroun et al., 1996)*. *Scheibel et al. (2002)* described the VAM to be the medial boundary for the subvastus region in total knee arthroplasty.

Previous relevant reports, in agreement with the present study, stated that VAM was a deep fascia stretched between the vastus medialis and the adductor magnus muscles overlying the saphenous nerve and femoral vessels *(Callander, 1939; Woodburne, 1978; Verta et al., 1984; Checroun et al., 1996; Tubbs et al., 2007; De Oliveira et al., 2009; Saranteas et al., 2011; Verma et al., 2013; Andersen et al., 2015)*.

Andersen et al. (2015) described the roof of the adductor canal as a continuous unbroken fascia with a thin (<0.5 mm) proximal and a thick aponeurotic distal part. This coincided with the present description of the proximal thin VFM and distal thick VAM, continuous type; however the attachment of the VFM to the femoral artery is not mentioned earlier.

In agreement with *Tubbs et al. (2007)*, the present study proved that the VAM was attached to the medial border of the vastus medialis muscle and also the VAM was wider proximally. Unlike what was proved in the present study, they reported that the VAM was rhomboid in shape and reached the lateral edge of the adductor magnus muscle.

The present study proved that the VAM represented an anatomical connection between the medial margin of both adductor magnus and adductor longus muscles and vastus medialis oblique (VMO) muscle, in agreement with *Bose et al. (1980) and Starkey and Ryan (2009)*. These findings may help to understand the role of the VAM as a pulley to increase the mechanical efficiency of the VMO muscle through the adductor magnus muscle over- activity.

The brownish striated appearance of the VAM in addition to being a connecting membrane between two muscles has raised a question. Does this membrane contain skeletal muscle fibres? So the skeletal muscle actin immunohistochemical staining of fresh cadaveric specimens was done to prove or disprove this. The presence of skeletal muscle fibres could neither be proved in the VAM nor in VFM as expressed by negative immunoreaction for the skeletal muscle actin.

Tubbs et al. (2007) reported that the majority of the VAM fibres was oblique from a superior to medial direction as proved in 6/ 40 specimens (15%) in the present study. However, they reported some VAM with proximal horizontal and distal oblique fibres. The present study proved the VAM middle fibres to be horizontal and the distal fibres to be oblique infero-laterally in 30/ 40 specimens (75%).

Checroun et al. (1996) reported that the proximal most point of the rounded adductor hiatus was consistently located at the level of the proximal edge of the VAM and the mean length of the VAM was 6 cm. Tubbs et al. (2007) reported that the mean length and width at the proximal, distal and midportion of the VAM were 7.6, 2.2, 0.5 and 1.7 cm respectively. The mean distance between the distal border of the VAM and the adductor tubercle was 10 cm *(Tubbs et al., 2007). Horn et al. (2009)* reported that the mean distance between the distal border of the VAM and the upper border of the patella was 10.25 cm.

The medial intermuscular septum (MIS) attaches to the medial lip of the linea aspera of the femur and its medial supracondylar ridge. Then it passes deeply between the vastus medialis and either the adductor longus or magnus to join the fascia lata either posterior *(Hall, 1990; Burnet et al., 2004; Standring, 2016)* or anterior (Rosse and Gaddum, 1997) to the sartorius. Woodbourne and Burkel (1994) suggested that the MIS splits to enclose sartorius.

It is apparent from the present histological features of the VM fascia, being thick layer of densely packed collagen fibres unlike the AM fascia, that it belongs to the MIS, in agreement with earlier reports (Woodbourne, 1978; Woodbourne and Burkel, 1994; Rosse and Gaddum, 1997; *Tubbs et al., 2007*). More proximally, at the attachment of VFM, the VM fascia was observed to be thinner as depicted in figure 17 of the present study.

Burnet et al. (2004) dissected eight thighs to investigate the sartorius fascial sheath and its role in sartorius protection from being invaded by soft tissue sarcomas arising nearby. They demonstrated that the sartorius is enclosed within its own fascial sheath derived from the fascia lata. In all eight thighs, at the level of the adductor canal there was evidence of a fascial envelope around sartorius.

In regard to the relation of the VAM to the MIS, *Hollinshead (1958)* described the VAM as a special thickening of the continuation of the medial intermuscular septum forming the roof of the adductor canal. *Romanes (1972)* reported that the MIS has become more indistinct and instead there is a well-marked thickening of a layer of deep fascia deep to sartorius, forming the VAM. *Woodburne (1978)* reported that the VAM ended in the medial intermuscular septum, in agreement with the present study. *Tubbs et al. (2007)* hypothesized that the VAM was derived from the medial intermuscular septum on the vastus medialis muscle.

The present study hypothesized that the VAM was derived from the tendinous fibres of the adductor magnus tendon which spread laterally, bridging the femoral vessels and saphenous nerve and ended in the VM fascia which belonged to the MIS. More proximally the VAM blended with the adventitia of the femoral vessels forming the VFM. The continuity and the same structural configuration of the VAM with the tendinous fibres on the surface of the adductor magnus muscle, in addition to being limited to the medial border of adductor magnus muscle, proved in the present study, may support the present hypothesis.

Even though the fenestrated VAM reported in 24/ 32 sides (75%) by *Tubbs et al. (2007)* was not encountered in the present study, the proved discontinuity between the VAM and the VFM in the present study may simulate the case depicted in figure 5 in their paper if the proximal part of the VAM was considered as the VFM.

It is reasonable to hypothesize that the VFM represents the proximal extension of the VAM, being continuous with it in 34/ 40 specimens (85%). However it is attached to the femoral artery instead of the adductor muscles. Both of which roofs a tunnel, deep to sartorius muscle, that contained in common the saphenous nerve and the femoral vessels. The thin transversely sectioned collagen bundles in its structure indicated that its fibres coursed longitudinally along the femoral artery. This collagen bundles direction and the thin VM fascia attached to it may be reasonable factors for little compressing or entrapment effect of the VFM.

In agreement with *Tubbs et al. (2007)* and *Verma et al. (2013)* the present study proved that the VAM was not pierced by the saphenous nerve or the descending genicular vessels or its saphenous branch. Instead the saphenous nerve constantly exits the vastoadductor tunnel through its distal aperture at which its entrapment does exist. In this regards *Saranteas et al. (2011)* reported that the saphenous nerve exits the adductor canal through its inferior foramina in 9 /11 (81.8%). In contradiction, other studies reported that the VAM was pierced by the saphenous nerve and the descending genicular vessels *(Luerssen et al., 1983; Romanoff et al., 1989; Checroun et al., 1996; Scheibel et al., 2002; Pendergrass and Moore, 2004; Horn et al., 2009 and Trescot et al., 2013).*

Even though the lower medial femoral cutaneous branch of the saphenous nerve constantly pierced the VAM in the present study, it did so in 10 /32 specimens (31%) in the study of *Tubbs et al. (2007)*. Entrapment of this nerve, at the piercing site, could explain medial knee pain with or without evident pain along the saphenous nerve distribution.

Medial knee pain caused by saphenous neuritis may imitate other clinical conditions particularly a medial meniscal tear or osteoarthritis *(Morganti et al., 2002).* Saphenous nerve entrapment is a difficult diagnostic clinical problem which may be solved by nerve tension, nerve course palpation and nerve conduction tests and diagnostic local anaesthetic injection *(Porr et al., 2013)*. Saphenous nerve entrapment may be presented by pain simulating vascular disorder of the leg *(Mozes et al., 1975)* and the pain was observed to increase with muscle exercise *(Sunderland, 1978).*

Being a site for neurovascular entrapment, *Checroun et al. (1996)* considered this membranous area of the VAM as the “danger zone”. They described it as a strong thickened fascial band. Despite the constant existence of the VAM proved in the present study, which is in agreement with *Tubbs et al. (2007)*, *Verta et al. (1984)* described the VAM as an abnormal band leading to acute femoral artery occlusion in young men following exercise. They stated that simple division of this abnormal band lead to restoration of the arterial blood flow to the leg.

The femoral artery may be subjected to acute occlusion at the adductor hiatus, or more proximally by the overlying VAM. The thick oblique and longitudinal three-layered collagen bundles in the structures of the VAM in addition to the contraction of the two attaching muscles may lay behind the neurovascular entrapment mechanism.

The present study proved the existence of perforating veins between the superficial veins and the femoral vein, piercing the VAM, in 840/ specimens (20%). Earlier they were reported in 61% of patients *(Tung et al., 1990)* and in 7/ 32 specimens (22%) *(Tubbs et al., 2007).*These authors opined that these communicating veins should be investigated with phlebography in patients with recurrent varicose veins.

Tubbs et al. (2007) have reported that cutaneous branch from the anterior division of the obturator nerve pierced the VAM in 2 /32 (6%). These findings could not be observed in the present study. Instead it proved to be one of the contents in the subsartorial space participating in the subsartorial plexus of nerves.

CONCLUSION

The subsartorial (adductor) canal included three fascial compartments, the vastoadductor and vastofemoral tunnels and subsartorial space. The two tunnels were roofed by two corresponding membranes which formed the subsartorial membranes. The subsartorial space lies superficially and is roofed by the deep layer of fascia lata split enveloping the sartorius muscle.

So under this concept the roof of the adductor canal would be the fascia on the deep surface of the sartorius muscle rather than the subsartorial membranes. The morphological and histological features of the subsartorial membranes, reported in the present study, may help the physicians to improve the management techniques of various clinical cases.

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الأغشية و الغرف تحت الخياطية فى القناة المقربة :دراسة شكلية، نسيجية و كيميائية نسيجية مناعية

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ملخص البحث

الخلفية: أصبح الوصف الدقيق للقناة المقربة و ما تحتويه من أغشية قضية لها أهمية جراحية و سريرية كبرى. إن احتباس الاوعية الدموية و الاعصاب داخل القناة المقربة قد يشبه عدة حالات سريرية تعانى من آلام فى الساق و الناحية الأنسية من الركبة و تبدو عليها أعراض قلة التغذية الدموية.

الهدف من الدراسة: كان الهدف من هذه الدراسة هو القاء الضوء على الغرف تحت الخياطية فى القناة المقربة و بيان الخصائص الشكلية، النسيجية و الكيميائية النسيجية المناعية لما تحتويه من أغشية.

المواد و الطرق: تم تشريح أربعين عينة فخد مأخوذة من 15 جثة محنطة و 5 جثث حديثة لأشخاص بالغين (15 من الذكور و 5 من الإناث) سعيا لتحقيق هذا الهدف. استخدمت صبغة ثلاثية الألوان و صبغة كيميائية نسيجية مناعية عن طريق استخدام أجسام مضادة وحيدة النسيلة مشتقة من الفئران لمادة أكتين العضلات الارادية لتوضيح تركيب هذه الأغشية.

النتائج: لوحظ وجود الغشاء المقرب المتسع ، فى الناحية القصوى و هو سميك خماسى الأضلاع و الغشاء الفخدى المتسع ، فى الناحية القريبة و هو رقيق ثلاثى الأضلاع كسقف لإثنين من الأنفاق اللفافى المناظرة لهما كما وجد الفراغ تحت الخياطية على سطح الغشائين السابقين. كان متوسط طول الغشاء المقرب المتسع و الغشاء الفخدى المتسع 7.9 و 7.8 سم على التوالى. امتد الغشاء المقرب المتسع عبر العضلة الأنسية المتسعة و العضلة المقربة الكبرى و غطى على العصب الصافن و فرعيه تحت الخياطية و الجلدى الفخدى الأنسى السفلى و الأوعية الدموية الفخدية و من واحد الى ثلاثة أعناق شريانية للعضلة تحت الخياطية و عنق شريانى الى العضلة الأنسية المتسعة و الأوعية الدموية الهابطة لمفصل الركبة. نشأ الغشاء المقرب المتسع من الألياف الوترية لوتد العضلة المقربة الكبرى و كان امتداده دائما الى الناحية الوحشية الأمامية. اخترق الغشاء المقرب المتسع بصفة دائمة الأعناق الشريانية للعضلة تحت الخياطية والفرعين الجلدى الفخدى الأنسى السفلى و تحت الخياطية للعصب الصافن. ثبت أختراق عنق شريانى للعضلة الأنسية المتسعة و الأوردة الثاقبة بين الأوردة السطحية و الوريد الفخدى للغشاء المقرب المتسع فى 8 من أصل 40 عينة. امتد الغشاء الفخدى المتسع بين الشريان الفخدى و العضلة الأنسية المتسعة و غطى على الأوعية الدموية الفخدية و العصب الصافن و كل من العصب و العنق الشريانى المغذيان للعضلة الأنسية المتسعة. تم اختراق الغشاء الفخدى المتسع بصفة دائمة بواسطة اثنين من الأعناق الشريانية للعضلة تحت الخياطية و أحيانا (فى 20 من أصل 40 عينة) بعصب موصل بين العصب الصافن و العصب الجلدى الفخدى الأنسى. أظهرت الصبغة ثلاثية الألوان للغشاء المقرب المتسع أنه يتكون من ثلاث طبقات متراكبة من حزم الكولاجين ظهرت فى اتجاهات مائلة و طولية و لها نفس التكوين الهيكلى و متواصلة مع اللفافة الوترية المغطية للعضلة المقربة الكبرى. كان الغشاء الفخدى المتسع مكونا من عدة حزم كولاجينية رفيعة و مستعرضة القطع. أثبتت الدراسة الكيميائية النسيجية المناعية للغشائين غياب ألياف العضلات الإرادية و ظهر ذلك عن طريق التفاعل المناعى السلبى.

الخلاصة: إن الخصائص الشكلية و النسيجية لهذه الأغشية تحت الخياطية قد تساعد الأطباء المعالجين لتحسين أسلوب معالجة حالات سريرية مختلفة.