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REGIONAL DIFFERENCES IN THE CELLULARITY AND VASCULARITY OF THE PATELLAR TENDON

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ABSTRACT

The patellar tendon (PT) attaches to the apex of the patella and tibial tuberosity. Its response to tensile forces is influenced by the distribution of fibroblasts and its vascularity. The vascularity and distribution of fibroblasts influence the tendons ability to repair microtears. Microtears of the PT result in patellar tendinopathy. There is however, paucity of data on the regional distribution of vascular and cellular elements in the PT which might explain why microtears occur in the posterior-proximal third. One hundred and two pairs of patellar tendons were obtained from postmortem specimens. Sections from the proximal, middle and distal third from the anterior and posterior lamina of 20 pairs of the patellar tendon (10 male, 10 female) were processed for microscopy to demonstrate the cellularity and vascularity of the tendon. The vascularity was highest in the middle third of the anterior lamina. The posterior lamina of the tendon was less vascular than the anterior lamina. The posterior lamina was more cellular than the anterior with the proximal third showing the highest number of nuclei. These findings indicate that the pre-patellar genicular anastomosis contributes significantly to the vascularity of the anterior lamina while the anastomosis located in Hoffa's fat pad may be less rich and thus resulting in lower vascularity for the posterior lamina. Lower vascularity implies less healing ability after microtears. Therefore, orthopedic surgeons should be aware of this precarious pattern of vascularity to the posterior lamina. The posterior lamina's high cellularity especially in the proximal third indicates that it may experience greater stress and via durotaxis more fibroblasts migrate to that region to produce more collagen fibers for resilience. The greater tensile stress experienced by the posterior-proximal third and its lower vascularity may explain why it is most susceptible to microtears.

Key words: Patella tendon, regional differences, cellularity, vascularity, patella tendinopathy

INTRODUCTION

The patellar tendon (PT) has its proximal attachment at the apex of the patella. It is distally attached to the tibial tuberosity (Standring, 2008). The tendon has been shown to have a rich blood supply from the genicular anastomosis (Pang et al., 2009). The blood flow to the tendon has been shown to increase with physical activity (Cook et al., 2005). The patellar tendon is covered by a paratenon that directs blood vessels to the substance of the tendon (Pang et al., 2009). This provides nutrition for the fibroblasts. These cells require a sustained vascular microcirculation to facilitate a turnover of amino acids in the collagen which is vital in maintenance of metabolic activity and healing of tendons (Fenwick, Hazleman and Riley, 2002).

Male patellar tendons have been shown to hypertrophy with exercise unlike in females (Sullivan et al., 2009). There is paucity of data on the difference in vascularity of the tendon between the sexes yet this may be useful in explaining the difference in adaptive response to physical exercise. The turnover of collagen in tendons has been studied in both sexes following exercise (Miller et al., 2007; Sullivan et al.,2009). These studies found the turnover to be lower in females.

According to Haraldsson et al (2005), female patellar tendons showed a lower tolerance to

tensile forces. The lower tolerance to stress led to earlier failure of the tendons (Haraldsson et al., 2005). However, the incidence of patellar tendinopathy is higher in males at a ratio of 6:1 (Levine, 2006). The posteromedial aspect of the proximal part of the patellar tendon is the most common site of patellar tendinopathy (Haraldsson et al, 2008). There are contradicting results regarding the tensile forces experienced in the anterior and posterior regions of the PT. According to Haraldsson et al (2008), the collagen bundles from the anterior region of the PT have demonstrated greater peak tensile stress levels than those from the posterior region. However, according to Almekinders, Jurrien and Paul (2002) the fascicles from the posterior region display greater peak tensile stress levels than those from the anterior region. Fibroblasts migrate to stiffer areas via durotaxis or mechanotaxis (Tschumperlin, 2013). Literature is however silent on the distribution of fibroblasts in the anterior and posterior regions of the patellar tendon in both sexes though it may help in understanding the differences in distribution of forces in the tendon and incidence of patellar tendinopathy.

MATERIALS AND METHODS

One hundred and two pairs of patellar tendon were used as study specimens. They were obtained by simple random sampling during autopsy procedures at the KNH mortuary and Nairobi City Mortuary. The autopsy specimens were collected from subjects without any obvious musculoskeletal pathology to the knee joint and above the age of 18 years. With the help of a pathologist at autopsy, any subjects with patellar tendinopathy, trochlear dysplasia, grossly malformed patella, multiple enthesophytes and trauma to the knee was excluded.

Tissue processing and photography

Thin slices of tissue were harvested from the proximal, middle and distal thirds of the tendon in the anterior and posterior aspect of the patella. The tissues were stained using hematoxylin and eosin to characterize the cellularity and vascularity of the patellar tendon. The slides were photographed using a Zeiss™ digital photomicroscope (Carl Zeiss AG, Oborkochen, Germany) at various magnifications and then copied onto a computer using Image J software (version 1.45s for Windows, National Institutes of Health, USA) for analysis.

Determination of vascularity of the Patellar tendon

Estimation of vascularity of the anterior and posterior lamina was done using morphometry. After taking photomicrographs of slides at x100 and x400, a cycloid grid was used to estimate the area of the blood vessels. Two fields of the same slide were used to obtain an average proportion of the vascular structures. The area was determined by multiplying the area of the grid (in pixels) by the magnification and the real width of the field in focus (in micrometers).

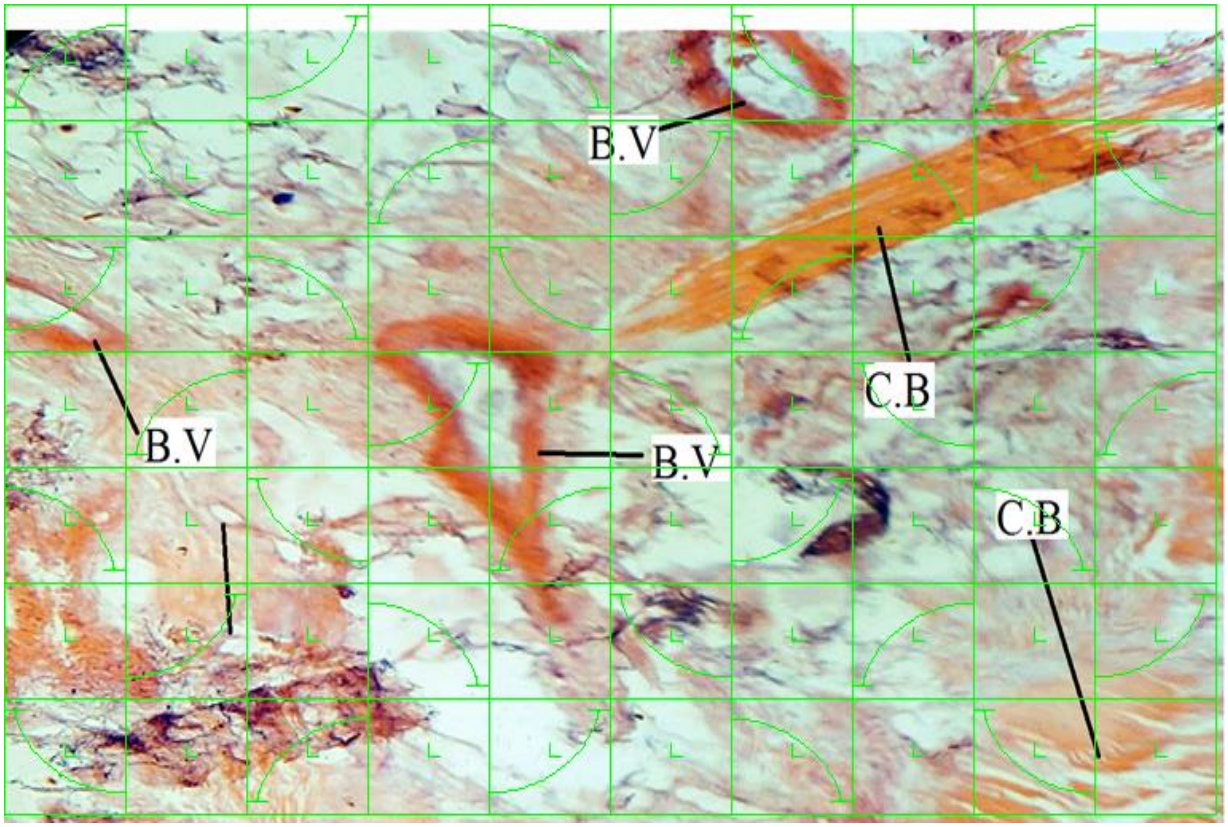

Figure 1: Photomicrograph of Patellar tendon vascularity overlaid with cycloid grid. H&E stain, X400, cycloid arc length L= 79.8668 pixels. B.V- Blood vessels, C.B- Collagen bundle.

Determination of cellularity of the Patellar tendon

Estimation of cellularity at the anterior and posterior lamina was done using morphometry. After taking photomicrographs of slides at x400, a cycloid grid was used to estimate the area of the cellular structures. Two fields of the same slide were used to obtain an average proportion of the cellular structures. he area was determined by multiplying the area of the grid (in pixels) by the magnification and the real width of the field in focus (in micrometers).

The observations and measurements were coded, tabulated and analyzed using SPSS version 21.0 (Armonk, NY: IBM Corp). The means, modes and standard deviations of the Insall-Salvati ratio in males and females was generated. Ethical approval for use of specimen obtained from autopsy material and conduction of the study was sought from the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee before the commencement of the study. Informed consent for the use of autopsy material was obtained from the next of kin of the deceased.

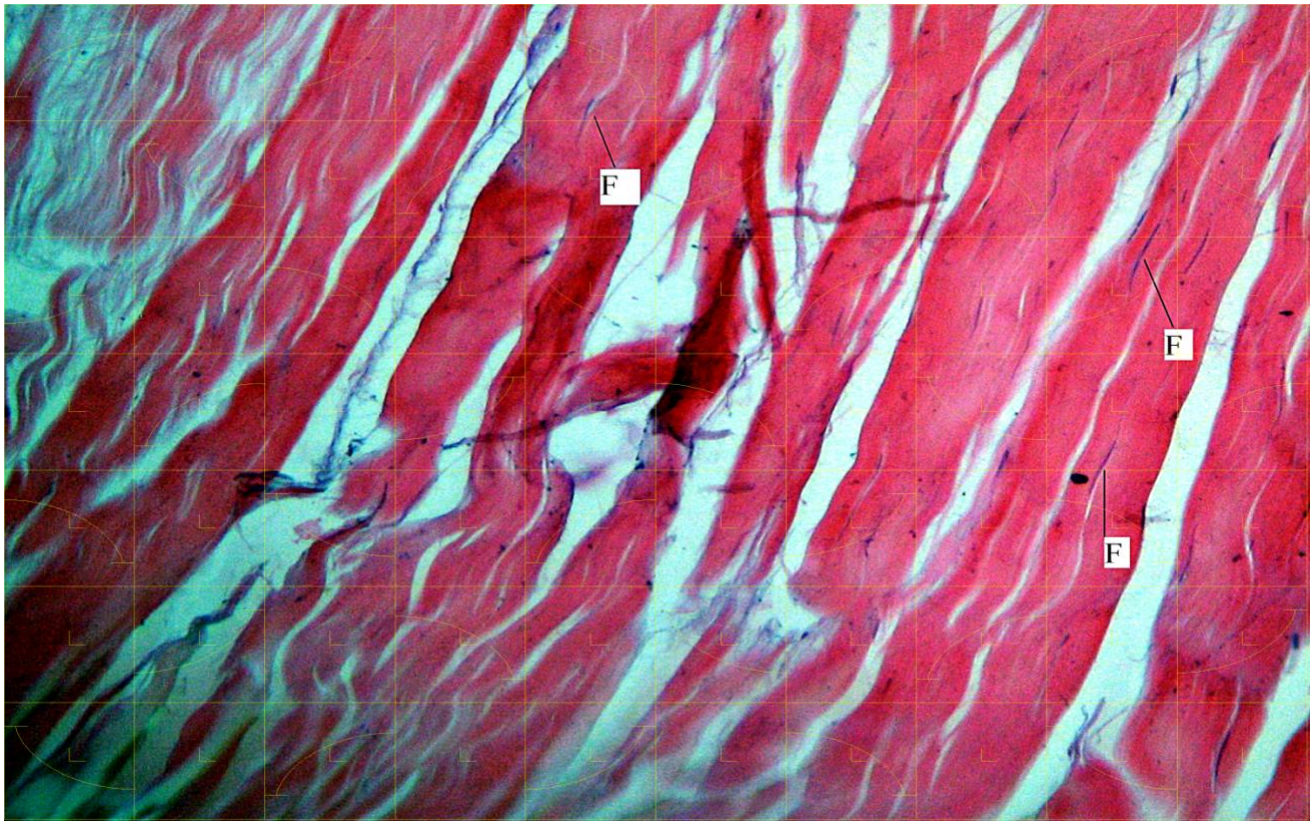

Figure 2: Photomicrograph of Patellar tendon cellularity overlaid with cycloid grid. H&E stain, X400, cycloid arc length L= 210.663 pixels

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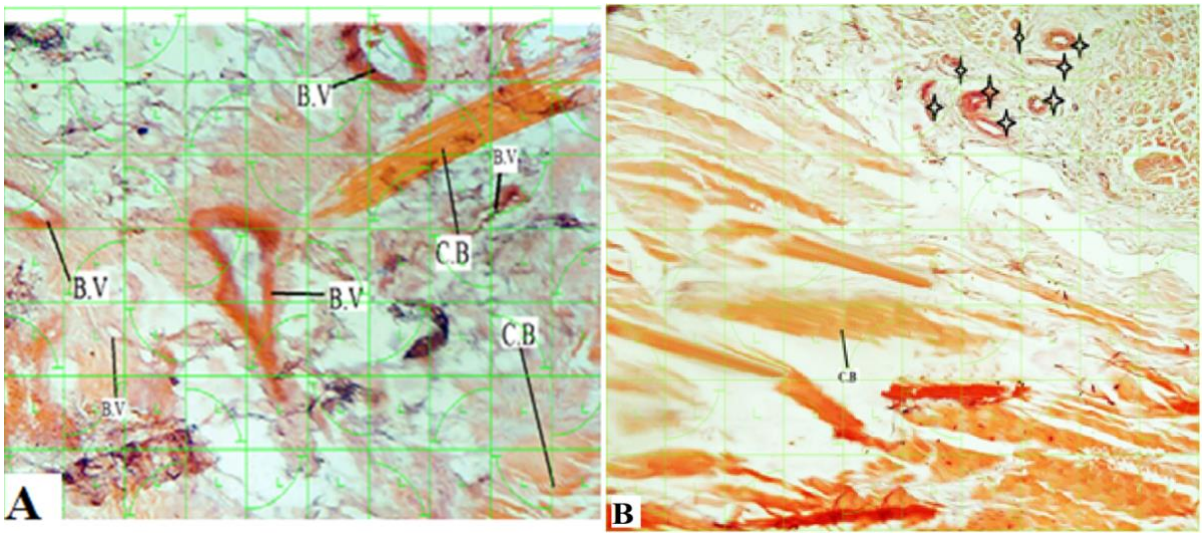
RESULTS

Vascularity of the patellar tendon



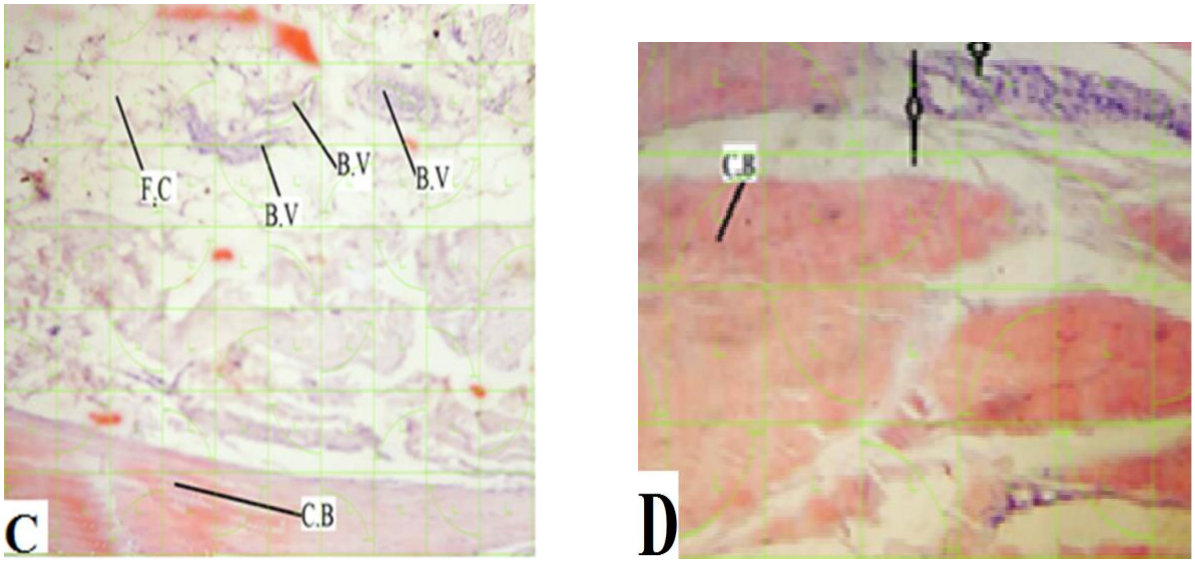
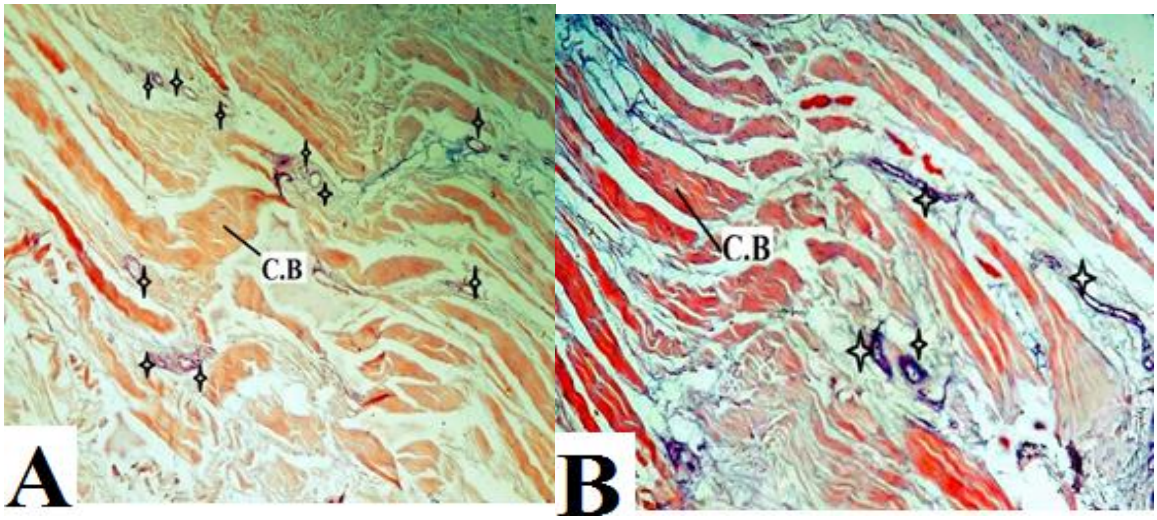


Figure 3: Photomicrographs showing the vascularity of the patellar tendon in males and females (proximal third). Figure 3-a: Transverse section of the proximal third of the posterior lamina of a right patellar tendon. The section is of a 28 year old male, it shows transversely arranged collagen bundles (CB) and blood vessels (B.V) in the infrapatellar fat pad (Hematoxylin and Eosin, X400); Note the 6 blood vessels/ mm^2 . **Figure 3-b:** Transverse section of the proximal third of the anterior lamina of a right patellar tendon. The section is of a 28 year old male, it shows transversely arranged collagen bundles (CB) and blood vessels (asterisks) in the endotendon. (Hematoxylin and Eosin X100); Note the 8 blood vessels/ mm^2 . **Figure 3-c:** Transverse section of the proximal third of the posterior lamina of a right patellar tendon. The section is of a 29 year old female, it shows transversely arranged collagen bundles (CB) and blood vessels in the infrapatellar fat pad (F.C) (Hematoxylin and Eosin, X100); Note the 5 blood vessels/ mm^2. **Figure 3-d:** Transverse section of the proximal third of the anterior lamina of a right patellar tendon. The section is of a 29 year old female, it shows transversely arranged collagen bundles (CB) and blood vessels in the endotendon. (Hematoxylin and Eosin X100); Note the 6 blood vessels/ mm^2.



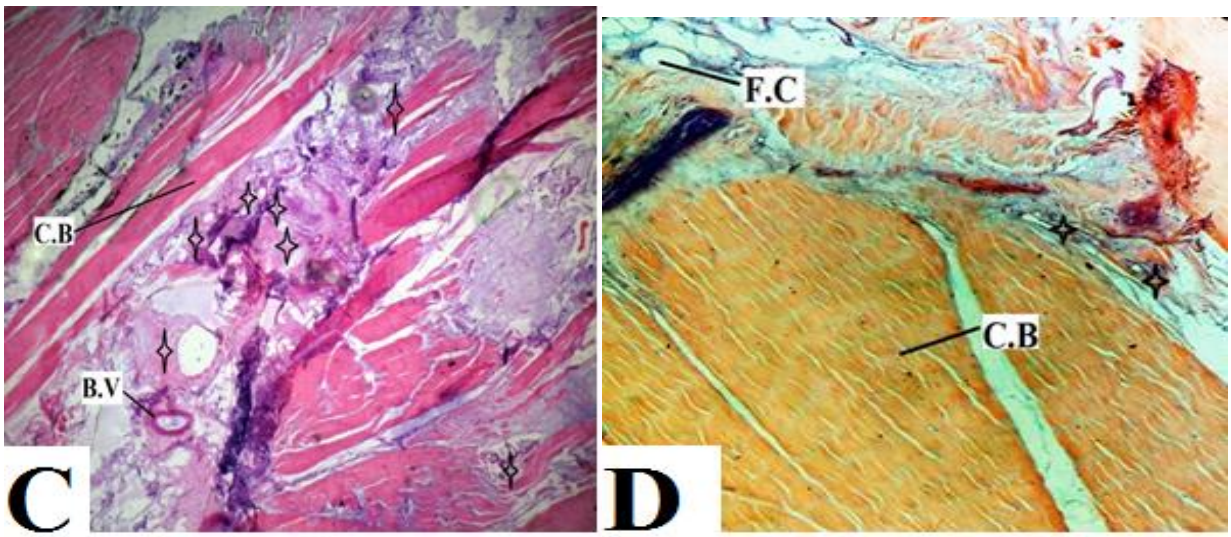
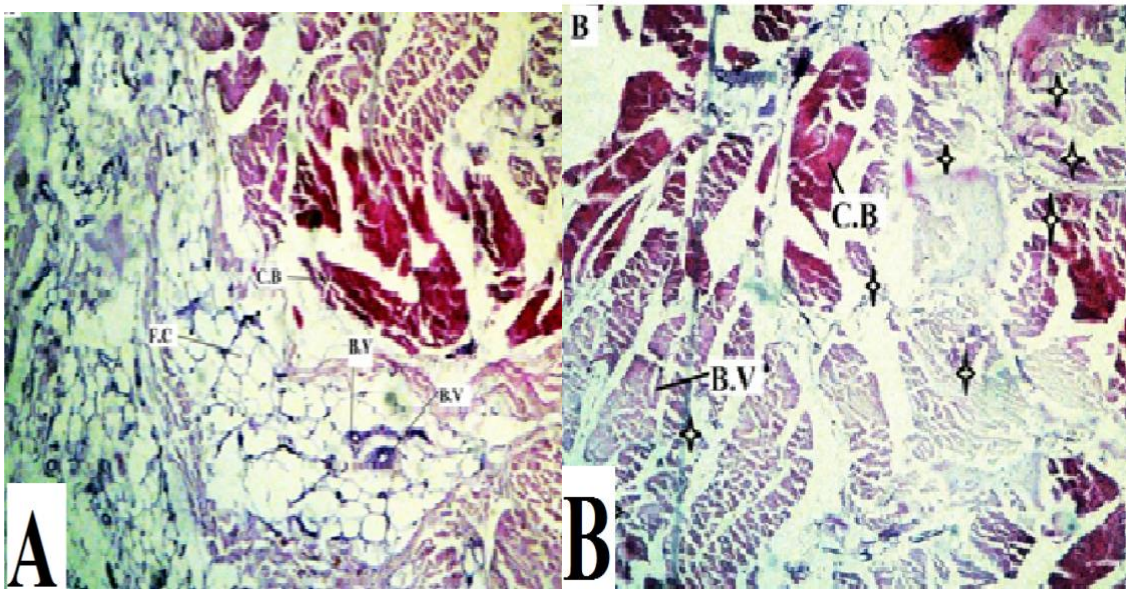


Figure 4: Photomicrographs showing the vascularity of the patellar tendon in males and females (middle third). **Figure 4-a:** Transverse section of the middle third of the anterior lamina of a right patellar tendon. The section is of a 25 year old male showing transversely arranged collagen bundles (CB) with blood vessels (asterisk *) interlaced between the collagen bundles. (Hematoxylin and Eosin X100); Note the 11 blood vessels/mm^2. **Figure 4-b:** Transverse section of the middle third of the posterior lamina of a right patellar tendon. The section is of a 25 year old male showing transversely arranged collagen bundles (CB) with blood vessels (asterisk *) in the region of the infrapatellar fat pad. (Hematoxylin and Eosin X100); Note the 4 blood vessels/mm^2. **Figure 4-c:** Transverse section of the middle third of the anterior lamina of a right patellar tendon. The section is of a 27 year old female showing transversely arranged collagen bundles (CB) with blood vessels running in the loose areolar tissue between the collagen bundles. (Hematoxylin and Eosin X100); Note the 8 blood vessels/ mm^2. **Figure 4-d:** Transverse section of the middle third of the posterior lamina of a right patellar tendon. The section is of a 27 year old female showing transversely arranged collagen bundles (CB) with blood vessels (asterisk *) in the region of the infrapatellar fat pad (FC). (Hematoxylin and Eosin X100); Note the 4 blood vessels/mm^2



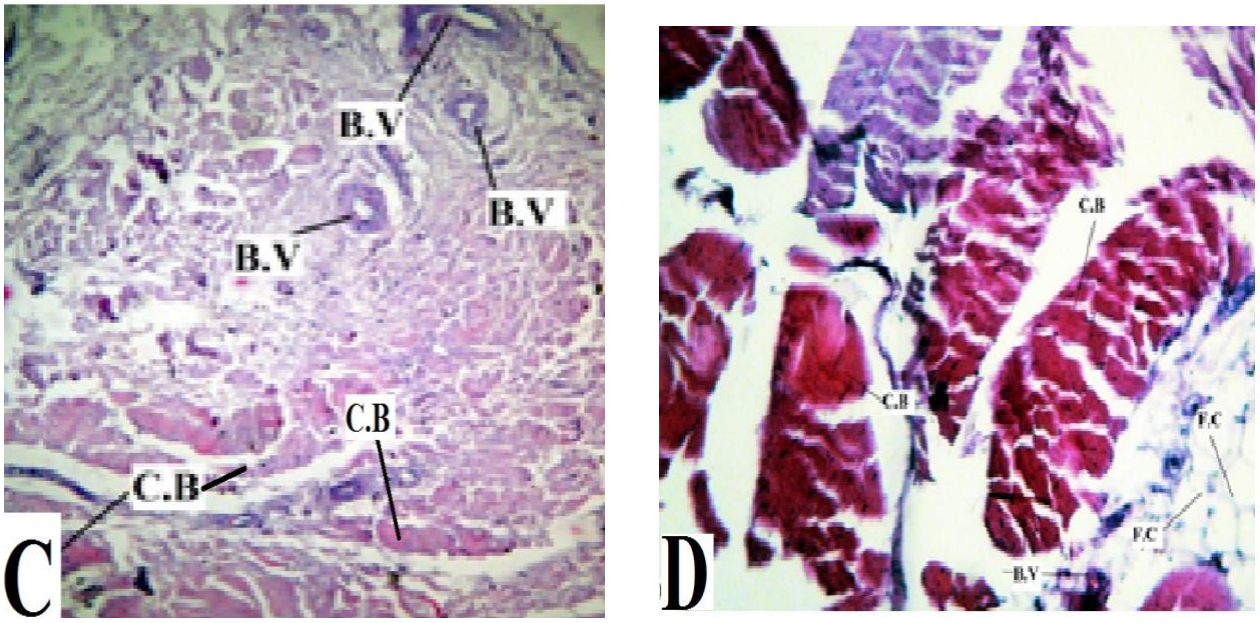
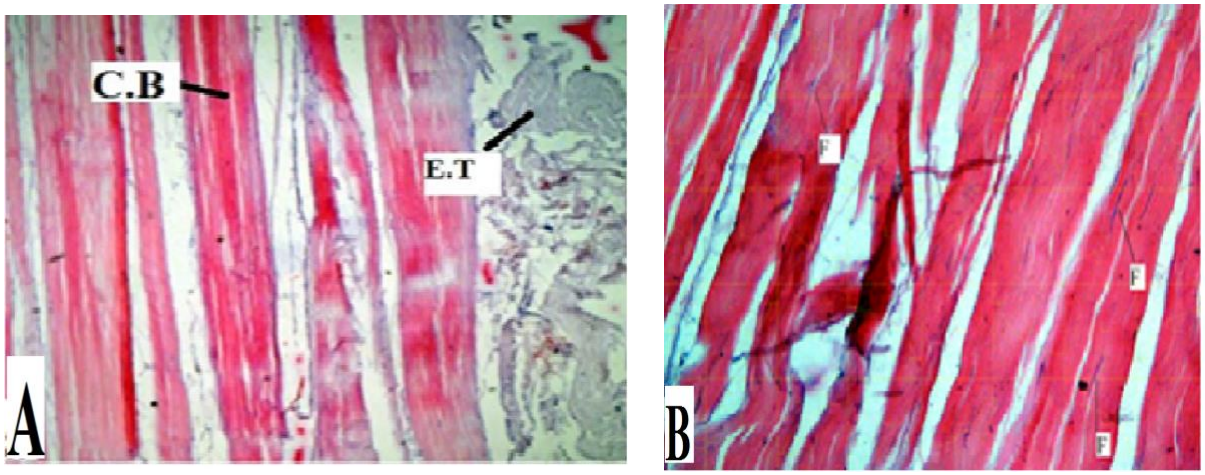


Figure 5: Photomicrographs showing the vascularity of the patellar tendon in males and females (distal third). **Figure 5-a:** Transverse section of the distal third of the posterior lamina of a right patellar tendon. The section is of a 29 year old male showing transversely arranged collagen bundles (CB) with blood vessels (asterisk *) in the region of the infrapatellar fat pad (F.C). (Hematoxylin and Eosin X100); Note the 4 blood vessels/mm^2 . **Figure 5-b:** Transverse section of the distal third of the anterior lamina of a right patellar tendon. The section is of a 29 year old male showing transversely arranged collagen bundles (CB) with blood vessels (asterisk *) interlaced between the collagen bundles. (Hematoxylin and Eosin X100); Note the 6 blood vessels/mm^2 . **Figure 5-c:** Transverse section of the distal third of the anterior lamina of a right patellar tendon. The section is of a 31 year old female showing transversely arranged collagen bundles (CB) with blood vessels (B.V) running in the endotendon. (Hematoxylin and Eosin X 100); Note the 4 blood vessels/mm^2. **Figure 5-d:** Transverse section of the distal third of the posterior lamina of a right patellar tendon. The section is of a 31 year old female showing transversely arranged collagen bundles (CB) with blood vessels in the region of infrapatellar fat pad (F.C). (Hematoxylin and Eosin X 100); Note the 3 blood vessels/mm^2

Cellularity of the patellar tendon



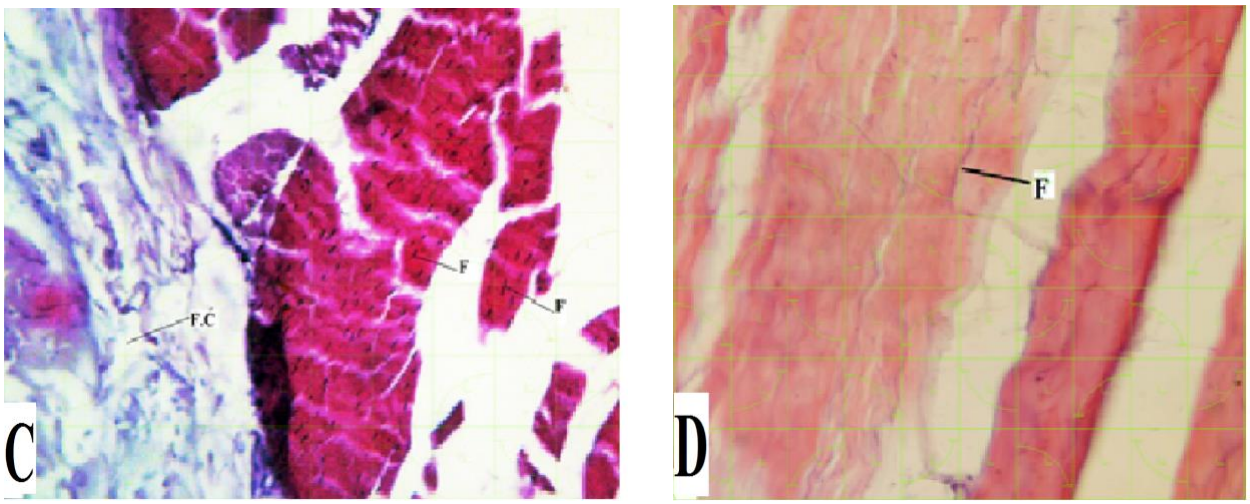
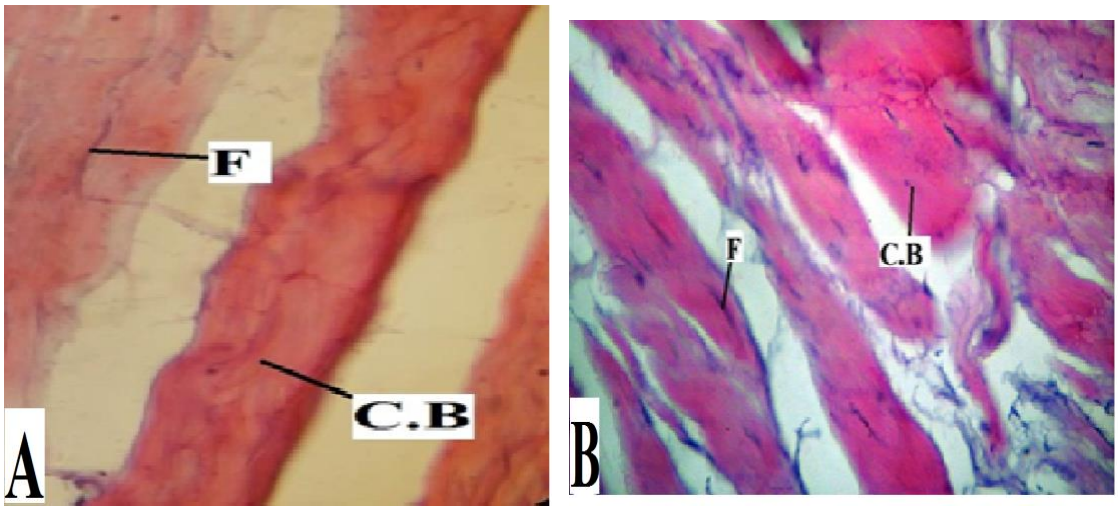


Figure 6: Photomicrographs showing the cellularity of the patellar tendon in males and females (proximal third). Figure 6-a: Longitudinal section of the proximal third of the anterior lamina of a right patellar tendon. The section is of a 25 year old male, it shows longitudinally arranged collagen bundles (CB) with the investing endotendon (E.T). (Hematoxylin and Eosin X400); Note the approximately 26 cell nuclei in the section. **Figure 6-b:** Longitudinal section of the proximal third of the posterior lamina of a right patellar tendon. The section is of a 25 year old male, it shows longitudinally arranged collagen bundles (CB) with numerous nuclei of cells akin to fibroblasts (F). (Hematoxylin and Eosin X400); Note the approximately 28 nuclei/mm^2. **Figure 6-c:** Longitudinal section of the proximal third of the posterior lamina of a right patellar tendon. The section is of a 26 year old female, it shows longitudinally arranged collagen bundles (CB) with numerous nuclei of cells akin to fibroblasts (F). Note the spaces representative of fat cells (FC) in the region of the infrapatellar fat pad. (Hematoxylin and Eosin X400); Note the approximately 20 nuclei/mm^2. **Figure 6-d:** Longitudinal section of the proximal third of the anterior lamina of a right patellar tendon. The section is of a 26 year old female, it shows longitudinally arranged collagen bundles (CB) with nuclei of cells akin to fibroblasts (F). (Hematoxylin and Eosin X400); Note the approximately 17 nuclei/mm^2



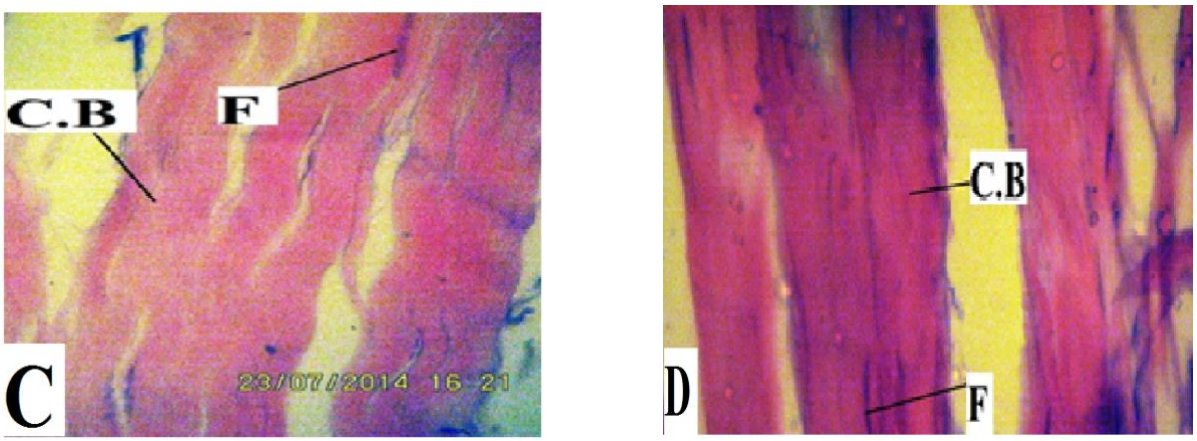
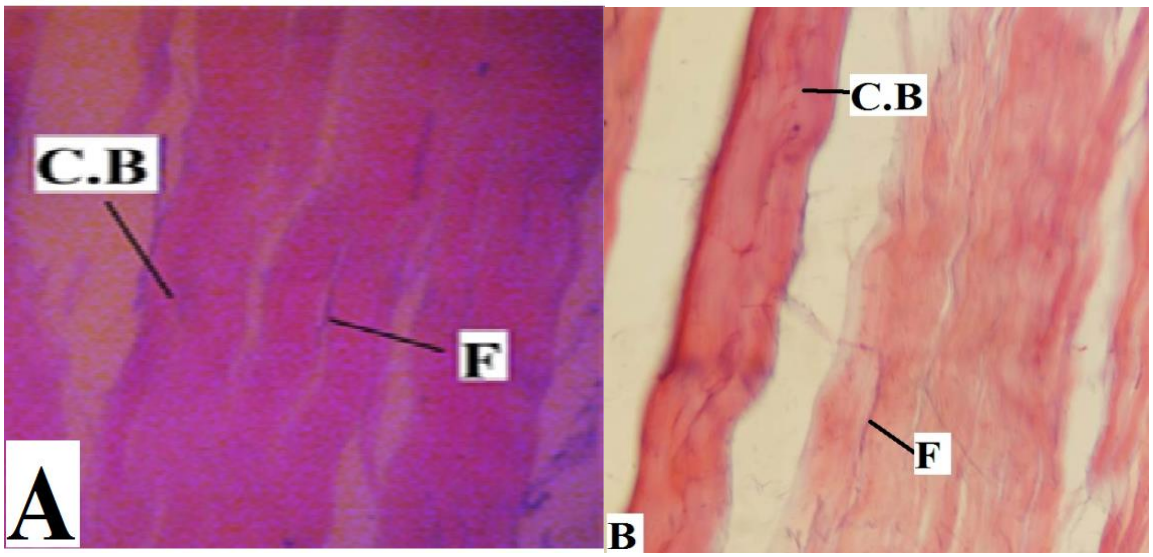


Figure 7: Photomicrographs showing the cellularity of the patellar tendon in males and females (middle third). **Figure 7-a:** Longitudinal section of the middle third of the anterior lamina of a right patellar tendon. The section is of a 32 year old male, it shows longitudinally arranged collagen bundles (CB). (Hematoxylin and Eosin X400); Note the approximately 21 nuclei/mm^2 of cells akin to fibroblasts (F). **Figure 7-b:** Longitudinal section of the middle third of the posterior lamina of a right patellar tendon. The section is of a 32 year old male, it shows longitudinally arranged collagen bundles (CB). Hematoxylin and Eosin X400); Note the approximately 24 nuclei/mm^2 of cells akin to fibroblasts (F). **Figure 7-c:** Longitudinal section of the middle third of the anterior lamina of a right patellar tendon. The section is of a 35 year old female, it shows longitudinally arranged collagen bundles (CB). (Hematoxylin and Eosin X400); Note the approximately 23 nuclei/mm^2 of cells akin to fibroblasts (F). **Figure 7-d:** Longitudinal section of the middle third of the posterior lamina of a right patellar tendon. The section is of a 35 year old female, it shows longitudinally arranged collagen bundles (CB). (Hematoxylin and Eosin X400); Note the approximately 27 nuclei/mm^2 of cells akin to fibroblasts (F).



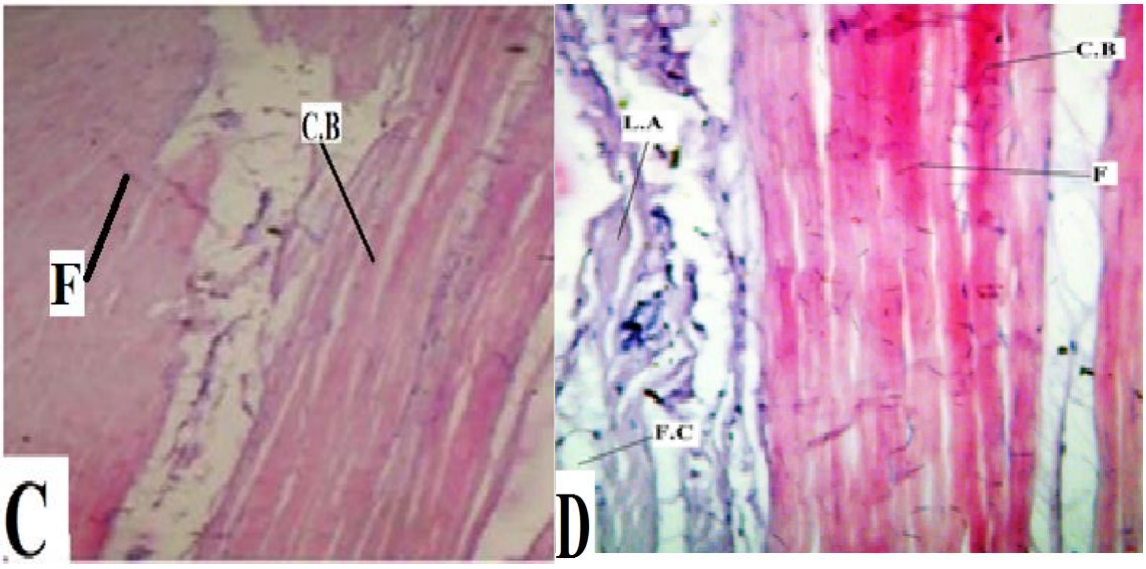


Figure 8: Photomicrographs showing the cellularity of the patellar tendon in males and females (distal third). Figure 8-a: Longitudinal section of the distal third of the anterior lamina of a right patellar tendon. The section is of a 22 year old male, it shows longitudinally arranged collagen bundles (CB). (Hematoxylin and Eosin X400); Note the approximately 22 nuclei/mm^2 of cells akin to fibroblasts (F). **Figure 8-b:** Longitudinal section of the distal third of the posterior lamina of a right patellar tendon. The section is of a 22 year old male, it shows longitudinally arranged collagen bundles (CB). (Hematoxylin and Eosin X400); Note the approximately 25 nuclei/mm^2 of cells akin to fibroblasts (F). **Figure 8-c:** Longitudinal section of the distal third of the anterior lamina of a right patellar tendon. The section is of a 23 year old female, it shows longitudinally arranged collagen bundles (CB). (Hematoxylin and Eosin X400); Note the approximately 20 nuclei/mm^2 of cells akin to fibroblasts (F). **Figure 8-d:** Longitudinal section of the middle third of the posterior lamina of a right patellar tendon. The section is of a 23 year old female, it shows longitudinally arranged collagen bundles (CB). (Hematoxylin and Eosin X400); Note the approximately 21 nuclei/mm^2 of cells akin to fibroblasts (F).

Table 1: Vascularity of the patellar tendon

*Average of luminal structures observed in 10 slides = Total number of vascular channels in anterior or posterior lamina in 10 slides/ 10

	ANTERIOR LAMINA		**POSTERIOR LAMINA**	
	MALE	**FEMALE**	**MALE**	**FEMALE**
Proximal 1/3	8	6	6	5
Middle 1/3	11	8	4	4
Distal 1/3	6	4	4	3

Table 2: Cellularity of the patellar tendon

*Average of nuclei observed in 10 slides = Total number of nuclei in anterior or posterior lamina in 10 slides/ 10

	ANTERIOR		**POSTERIOR**	
	MALE	**FEMALE**	**MALE**	**FEMALE**
Proximal 1/3	26	17	28	20
Middle 1/3	21	23	24	27
Distal 1/3	22	20	25	21

DISCUSSION

Patellar tendinopathy is associated with microtears in the patellar tendon. It is more prevalent in males than females at a ratio of 6:1 (Levine, 2006). Patellar tendinopathy occurs commonly in the posteromedial part of the proximal aspect of the patellar tendon **(**Maffulli, Jason and Louis, 2003). This region was found to be the most cellular in our study (Fig.6b). Accordingly, fibroblasts are expected to be numerous in regions experiencing great tensile stress (Lo, 2000; Tschumperlin, 2013). This finding agrees with the results obtained by Almekinders, Jurrien and Paul (2002) who found that the posterior region of the patellar tendon experiences greater tensile stress than the anterior region. However, this contradicts the results of Haraldsson et al (2008) who found that the anterior collagen fascicles experienced greater tensile stress than the posterior collagen fascicles.

The posterior region of the patellar tendon was found to be less vascular than the anterior region (Fig. 3b &c, fig.4b &d, 5b &d). It receives its blood supply predominantly via the infrapatellar fat pad (Nemschak and Michael, 2012). The anterior region receives direct blood supply from the anastomosis between the superior lateral and medial, inferior lateral and medial, and the anterior tibial recurrent artery (Pang et al., 2009). The anastomosis is richest at the middle third which is commensurate with our findings (Fig 4a & c).

There was no difference in the vascularity and cellular distribution of the tendon in both sexes. This implies that intrinsic factors of the tendon are similar in both sexes and may not be used to explain the difference in prevalence of patellar tendinopathy between the sexes.

Vascularity of the patellar tendon

The vascularity of the posterior region of the patellar tendon was relatively less (Fig. 3a &c, fig.4b &d, 5a &d). The blood supply to the posterior part of the patellar tendon (PT) is by the anastomosis in the infrapatellar fat pad (Hoffa's fat pad) which plays a vital role in the dissipation of stress exerted on the patellar tendon (Nemschak and Michael, 2012). The PT fibers penetrate the fat pad and any irritation to the neural structures contributes to the pain in patellar tendinopathy.

The studies by Soldado et al (2002) and Pang et al (2009) showed a rich arterial anastomosis in the anterior lamina where the middle third had the richest vascularity. The results of the present study are in agreement with this (Fig. 5a). The study by Cook et al (2005) also showed no significant difference in the blood flow during physical exercise between the sexes. The results from the present study showed no gender difference in the vascularity of the patellar tendon. This suggests that the nutrition to the tendon may be similar in both genders and vascularity may not be used as an explanation for the difference in prevalence of patellar tendinopathy between the sexes.

Cellularity of the patellar tendon

The results of this study depict more cellularity in the posterior region than the anterior region proximo-distally in both sexes. Fibroblasts are mechanotransducers which means that they convert mechanical stimuli into biochemical activities such as collagen synthesis. Accordingly, a high number of fibroblasts are expected in a region which experiences great tensile forces due to the phenomenon of durotaxis or mechanotaxis (Lo, 2000; Tschumperlin, 2013). This may be important for tendon repair healing (Massoud AND Ibraheem, 2013).

The studies by Almekinders, Jurrien and Paul (2002) and Docking et al (2013) found that the posterior region of the patellar tendon experienced greater tensile forces than the anterior region. Their findings contradict

Haraldsson et al (2008) who found the anterior region of the tendon experiences greater tensile forces than the posterior region. The results of this study thus agree with the findings of Almekinders, Jurrien and Paul (2002). In the present study, the proximal third of the posterior lamina showed a high number of spindle shaped nuclei resembling fibroblasts (Fig.6b). Therefore, this suggests that the posterior region experiences more tensile forces as evidenced by the high number of nuclei akin to fibroblasts noted in the present study.

The present study showed that the cellular distribution was almost similar in both sexes which is in agreement with Hashemi, Naveen and James (2005) who found that the mechanical properties of the patellar tendon were correlated to mass density and were independent of sex. However, this contradicts the findings by Onambe'le, Katherine and Stephen (2007) who found gender-specific in vivo measurement of the structural and mechanical properties of the human patellar tendon. Male patellar tendons have been shown to hypertrophy with exercise though this was not observed in females (Sullivan et al., 2009). The turnover of collagen in tendons has been found to be lower in females following exercise (Miller et al., 2007; Sullivan et al., 2009). Therefore, since no gender difference was observed in the cellular distribution of the patellar tendon (PT), this suggests that probably fibroblasts of the PT are influenced by sex hormones and this affects their response to physical exercise.

A study by Karageanes, Kim and Zenos (2000) showed an association of the menstrual cycle with the laxity of the anterior cruciate ligament in adolescent female athletes which was in agreement with the findings of Deie et al (2002) and Pollard (2006). According to Faryniaz et al (2006), estrogen receptors are present in the fibroblasts of the anterior cruciate ligament. A study by Eiling et al (2007) showed an influence of estrogen on musculotendinous stiffness and knee laxity during menopause. Thus, future studies should characterize estrogen and androgen receptors in the fibroblasts of the patellar tendon may aid in explaining the difference in response to tensile forces between the sexes.

In conclusion, results from our study show that there are no differences in the vascularity and cellular distribution of the patellar between the sexes. It was however noted that the posterior lamina is more cellular and less vascular. Data on the prevalence of patellar tendinopathy amongst Africans would also be useful. Future studies should characterize estrogen and androgen receptors in the fibroblasts of the patellar tendon

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