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Comparative Investigation of the Fetus and Adult Joint Ligament in the Knee and Elbow with Structural Levels

Investigación Comparativa del Ligamento Conjunto de Rodilla y Codo con Niveles Estructurales en Fetos y Adultos

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SUMMARY: The aim of this study was to examine and compare fetal and adult knee and elbow joint ligaments and determine histologically how these ligaments change over time. In addition, the images of fetal and adult joint ligaments were examined with magnetic resonance imaging (MRI). This study was conducted on 10 male fetus ranging from ages 14 to 17.5 weeks, two adult male cadavers obtained from Gazi University Faculty of Medicine Department of Anatomy and MR images of the knee and elbow regions of 10 male adults obtained from Atatürk Educational and Research Hospital between 2009 and 2011. In the present study, the sections taken from knee and elbow of ten 14–17.5 week old fetuses and the ligaments of tissue taken from the knee and elbow of two male cadavers using the same method of dissection were monitored. After monitoring tissue, microtome sections taken from paraffin-embedded structures were stained using the Masson-Trichrom and Orcein-Picroindigocarmine staining method. These sections were examined under a microscope and photographed. The images of 17 week old fetuses and the knee and elbow of the adults were obtained with MRI. The differences detected between adult and fetus ligaments consisted of fibroblast density and collagen thickness, density and waves. Although the fetus ligaments were not seen sufficiently with 1.5 Tesla (T) MR, they were seen very clearly with 3 T MR. Structural differences between adult and fetal ligaments revealed in histological and MRI images.

KEY WORDS: Knee ligaments; Elbow ligaments; Magnetic resonance; Collagen fibers; Fibroblast.

INTRODUCTION

Joints carry the load outside of the body as well as the body weight. Knee and elbow joints become more vulnerable to impact from the outside due to their structural features and functions. So, degeneration developing over time and as a result, diffuse joint pain occur. Therefore, it constitutes an important part of reasons for applying in orthopedic clinics. The structures, which belong to these joints, have attracted people's attention from the very earliest times until today. Information on joints extends up to GALEN's (Claudius Galenus, Calinus-A.D. 130-200) period. Galen made the identification of the ligaments of joints and gave information about their structures in his work, that he examined in sports injuries (Galen, 1968; Snook, 1983). The studies on joint and its structures are continuing and today pathologies belonging to these ligaments can be much more readily detected as a result of the increase in imaging techniques (Brown & Semelka, 2010).

There are several factors holding these bone structures together and providing joint stability. The most prominent of these factors are joint capsule which is the structure wraping the outside ends of the bones involving the joint and fibrous structures (ligaments) combining the bones forming the joint. They show improvement proportional to the force that the joint faced. Therefore, joint capsules and ligaments are weaker where the force is lesser in the joint, and these structures are stronger where the force is greater in the joint (Williams *et al*., 1995, 1989; Snell, 1995).

The ligaments are named according to their existence inside or outside the articular capsule as internal ligaments and external ligaments. These ligaments in the human body are very strong structures. The most important reason for this is the collagen fibers they contain. There are cruciate ligaments between collagen molecules. During the development period,

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the number and quality of cruciate ligaments between collagen molecules increase. At the same time, the diameter of the collagen fibers also enlarges. These cause an increase in the strength of the ligament (Junqueira & Carneiro, 2005).

Ligaments are the tissues with less vascularization and composed of collagen fibers arranged in parallel. About 20% of the volume fraction is formed by fibroblasts. It has a dense matrix outside the fibroblasts. Seventy per cent of matrix consists of water and 30% consists of collagen, ground substance and elastin. Collagens provides high tensile strength. Seventy to 80% of this 30% is formed by collagens (Kelly *et al*., 1984).

Although, several studies regarding joint development have been performed, there are not enough works available on the development of articular ligaments (Merida-Velasco *et al*., 1997). It has been reported that, the studies performed were conducted more on animals, the examination was difficult with dissection or radiological imaging methods as the human embryo and fetus are small (Hounnou *et al*., 2003).

In this study, it was aimed to compare the articular ligaments obtained from fetal knee and elbow joints with those obtained from the adult cadavers with serial sections and dissection methods at structural level and determine how these ligaments underwent a histological change over time. In addition, the images of articular ligaments in fetus and adult patients were studied to anatomically evaluate with MRI methods.

MATERIAL AND METHOD

This study was conducted on 10 male fetuses ranging from ages 14 to 17.5 weeks, two adult male cadavers obtained from Gazi University Faculty of Medicine Department of Anatomy and MR images of the knee and elbow regions of 10 male adults obtained from Atatürk Educational and Research Hospital between 2009 and 2011. Ethical Approval was granted by the Research Ethic Committee of Gazi University Faculty of Medicine. Fetuses were fixed in 10% formaline solution for at least 6 months. The knee and elbow joint regions of these fetuses were serially sectioned by cutting horizontally by binding from the proximal and distal regions including ligaments contained herein for the protection of the natural anatomical structures.

In Faculty of Medicine, Department of Anatomy, two adult male cadavers were fixed in 10% formalin solution for at least 1 year. The knee and elbow regions of these cadavers were dissected and all of the ligaments belonging to knee and elbow joints in these regions were named individually. In addition, MRI images belonging to the region of the elbow and knee of 17-week male fetus and10 adult males applied to Ataturk's Hospital, Department of Radiology were evaluated.

Each of the ligaments taken from adult cadavers as well as the section taken from fetuses were separately placed in jars with 10% formalin solution. After this fixation procedures, they were subjected to the decalcification process for removing the tissues from the bone structures. Formic acid solution was used for this process.

All materials were dehydrated in an increasing ethanol series, infiltrated with xylene and embedded in paraffin. Transverse sections of 5 μm thickness were cut and dewaxed. After this process, the materials were taken to paraffin bath and embedded into molds.

The preparations obtained with staining with both Masson-Trichrome and Orcein-Picroindigocarmine, were examined histologically with Leica DM 4000 B Germany photomicroscopy with 1.25x10, 10x10, 40x10 magnification rates.

Four of the 3-dimensional images of knee and elbow joints obtained by using PACS program software (Extreme PACS, Ankara) 3 Dimensional proton density (3DPD) sequences taken in the coronal and sagittal planes for knee and elbow joints in 10 adult male with 1.5 Tesla MRI unit (Philips Achieva, The Netherlands) for magnetic resonance imaging, were taken. The images of the fetus were obtained with 3 Tesla MRI system (Siemens Timtriosyste 15 channel knee coil).

RESULTS

In the images of the right knee's patellar ligament of the adult cadaver group stained with Masson-Trichrome, it was noticed that the collagen fibers were lying parallel to each other, forming tightly packed sheaves in cylindrical appearance. Diameters and arrangement of the collagen fibers,the size of the fibroblast cells differ according to the knee ligaments in fetus (Fig. 1A, B). It was noticed that, adult ligament structure had the arrangement in the form of a completely regular dense connective tissue.

It was observed that the fiber density and fibroblasts forming the fiber were more in number in the fetuses but the diameter of collagen fibers and the diameter of fibroblast cells were significantly smaller than in the adults.

It has been observed in the images of right knee's fibular collateral ligament of adult cadaver group stained with Masson trichrome that, there were the bundles consisting of collagen fibers with thinner diameter compared collagen fibers of patellar ligament which is in parallel to each other, loose connective tissue between fibers (Fig. 1C).

It has been observed in the images of right knee's fibular collateral ligament of seventeen week fetus group stained with Masson trichrome, the layer consisting of chondroblasts inside and chondrogenic cells outside, which created resource for the articular cartilage and the cartilage cells in the periphery attracted attentions (Fig. 1D, E). It was noticed that, lacunas of chondrocytes forming articular cartilage did not fully develop, did not have a full structure of cartilage cells with spindle shape and extensions yet. The cells were wrapped circumferentially by extracellular matrix (Fig. 1F). In the same section of the knee joint, ossification region in the corresponding location of the distal part of femur and the proximal part of the tibia was seen. Bone trabeculae which are irregular and anastomosing with each other and bone marrow cells among them were noticed (Fig. 2A). In the same section of the knee joint tibial collateral ligament was ongoing and inserted with joint capsule, and right next to it, the transverse section of tibial nerve surrounded by connective tissue sheaths called perineurium and epineurium were seen (Fig. 2B). Again in this section, it has been observed that, fibular collateral ligament consisted of collagen fibers extending parallel to each other and oval shaped fibroblasts synthesizing them. When the diameter of the collagen fibers and shapes, organization and size of fibroblasts cells were compared to collagen and fibroblasts in adult ligaments, significant differences were seen (Fig. 2C). Density of collagen and fibroblasts was observed more than the adult ligaments.

It has been observed in the images of left elbow anular ligament of radius of seventeen-week fetus group stained with Masson trichrome, the collagen density where anular ligament of radius adhered to the bone were lesser intense compared to collagens of this ligament in other regions (Fig. 3F).

It was noticed in the sections of right elbow'sulnar collateral ligament of adult cadaver group stained with Orcein-Picroindigocarmine that, collagen fibers which arranged as regular dense connective tissue had more undulate structure and stained as navy-blue color by taking Indigo blue inside the Orcein-Picroindigocarmine (Fig. 2D).

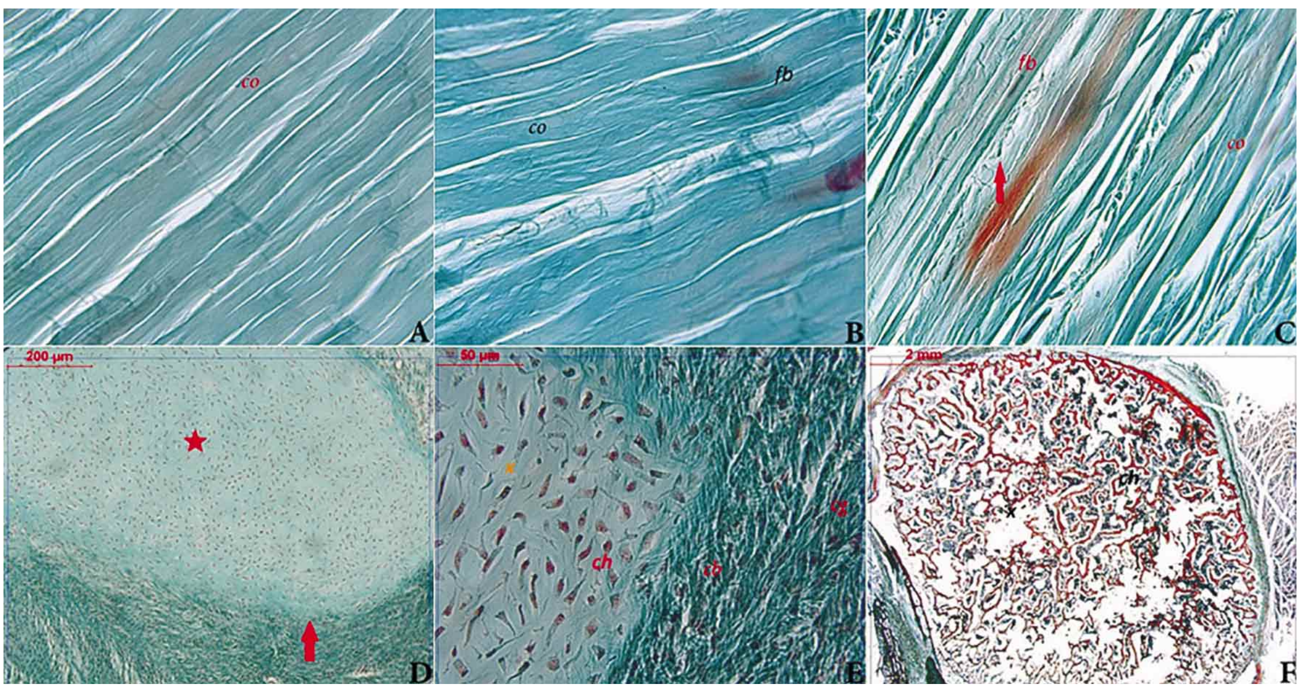

Fig. 1. Right knee patellar ligament of adult cadaver group; aligned parallel bundles of collagen fibers (co) (Masson-Trichrome staining, x40) (A). Right knee patellar ligament of adult cadaver group; parallel stretched bundles of collagen fibers (co), fibroblasts (fb) (Masson-Trichrome staining, x40) (B). Fibular collateral ligament of the right knee of adult cadaver group; loose connective tissue (–>), collagen fibrils (co), fibroblasts (fb) among them (Masson-Trichrome staining, x40) (C). The right knee joint of 17-week-old fetus; articular cartilage (x) and the surrounding outer layer is seen (⇑) (Masson-Trichrome staining, x10) (D). Right knee joint of 17-week-old fetus; Chondrocytes (ch), extracellular matrix (x), the surrounding floor outside chondrogenic cells (cg), chondroblasts (cb) inside (Masson-Trichrome, x40) (E). Right knee joint of 17-week-old fetus; needle forms, chondrocytes (ch) with the extensions and intercellular matrix (x) (Masson-Trichrome, x10) (F).

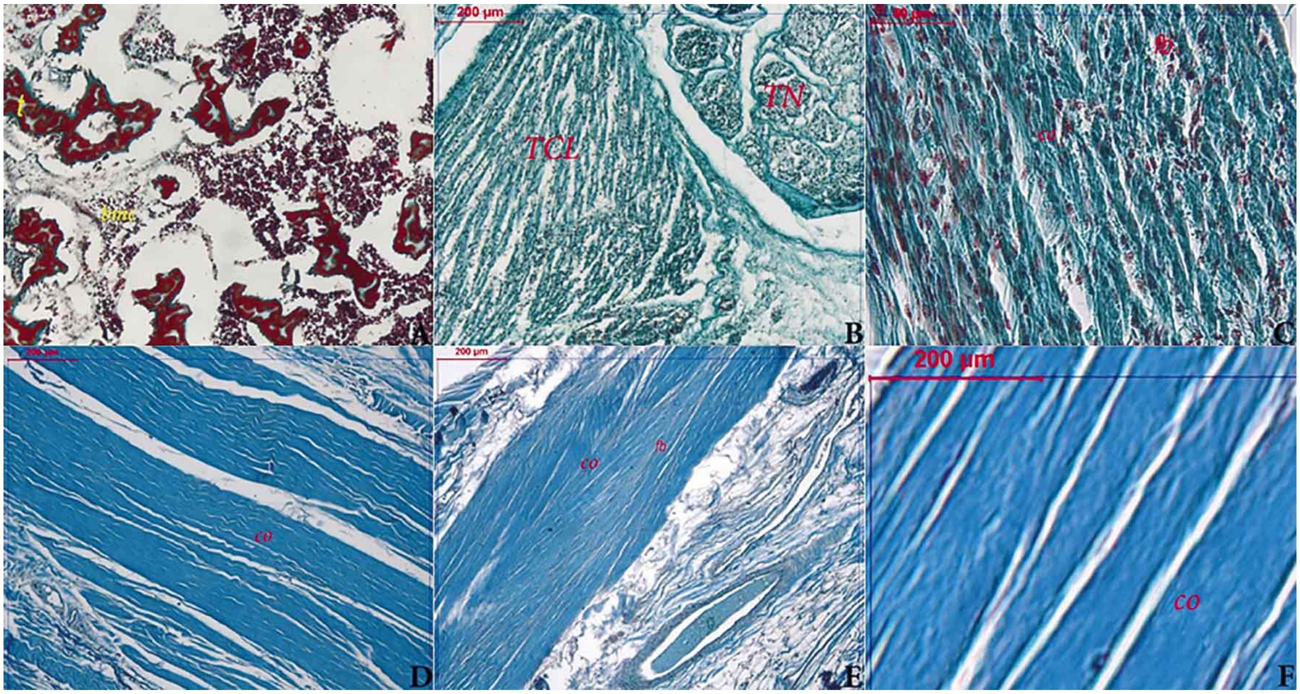

Fig. 2. Right knee of 17-week-old fetus; Ossification is seen in the corresponding location to the distal femur and proximal tibia region. Bone trabeculae (t) and bone marrow cells (bmc) among them (Masson-Trichrome staining, x40) (A). Right knee of 17 weeks old fetus; cross-section of the tibial collateral ligament (TCL) and the tibial nerve (TN) (Masson-Trichrome staining, x10) (B). Right knee joint of 17-week-old fetus; collagen fibrils (co) which form the fibular collateral ligament and fibroblasts (fb) which synthesis is seen in enlargement (Masson-Trichrome staining, x40) (C). Right elbow ulnar collateral ligament of adult cadavers group. Parallel collagen fibrils (co) (Orcein-Picroindigocarmine staining, x10) (D). Left elbow radial collateral ligament of adult cadaver group. Tightly packed bundles of collagen fibrils (co) and fibroblast (fb) nuclei (Orcein-Picroindigocarmine staining, x10) (E). Right knee tibial collateral ligament of adult cadaver group. collagen fibril (co) bundles are seen in the same direction, parallel to each other and cylindrical (Orcein-Picroindigocarmine staining, x40) (F).

It was noticed in the sections of left elbow's ulnar collateral ligament of the same group stained with Orcein-Picroindigocarmine that, collagen fibers formed tight bundles in a similar way with ulnar collateral ligament, its enviroment was surrounded by loose connective tissue. It has been observed that, fibroblast nucleicolored in red-brown by taking Orcein inside the Orcein-Picroindigocarmine (Fig. 2A).

It was observed in the sections of right knee's ulnar collateral ligament of adult cadaver group stained with Orcein-Picroindigocarmine that, collagen fibers forming the ligaments were thicker than the diameter of those in upper extremities (Fig. 2F). It was observed in the sections of right knee's anterior cruciate ligaments and left knee's posterior cruciate ligaments of the same group stained with Orcein-Picroindigocarmine that, the collagen fibers of the anterior cruciate ligament presented very dense, quite tightly and in nested form (Fig. 3A). It was detected that, the collagen fibers of the posterior cruciate ligaments similarly with anterior cruciate ligaments (Fig. 3B).

In the sections of right elbow's triceps brachii tendon of adult cadaver group stained with Orcein-Picroindigocarmine it was observed that, collagen structures and fibroblasts stained in the same color with elbow ligaments. Structurally, the fibroblasts belonging to these tendon were observed as long and thin (Fig. 3E).

In the sections of the right knee's cruciate ligaments of fifteen-week fetus group stained with Orcein-Picroindigocarmine, it was observed that the ligaments began to take form, fibroblasts did not fully differentiate, the structure of regular dense connective tissue did not fully develop yet (Fig. 3C, D).

The 1.5 T MR images of the ligaments of the knee and elbow joints belonging to adult humans were obtained in the coronal and sagittal planes. In the image section of the knee joint in the coronal planes it was observed that fibular collateral ligament did not have connection with lateral meniscus (Fig. 4A). In the image section of the knee joint in the sagittal plane, anterior cruciate ligament was

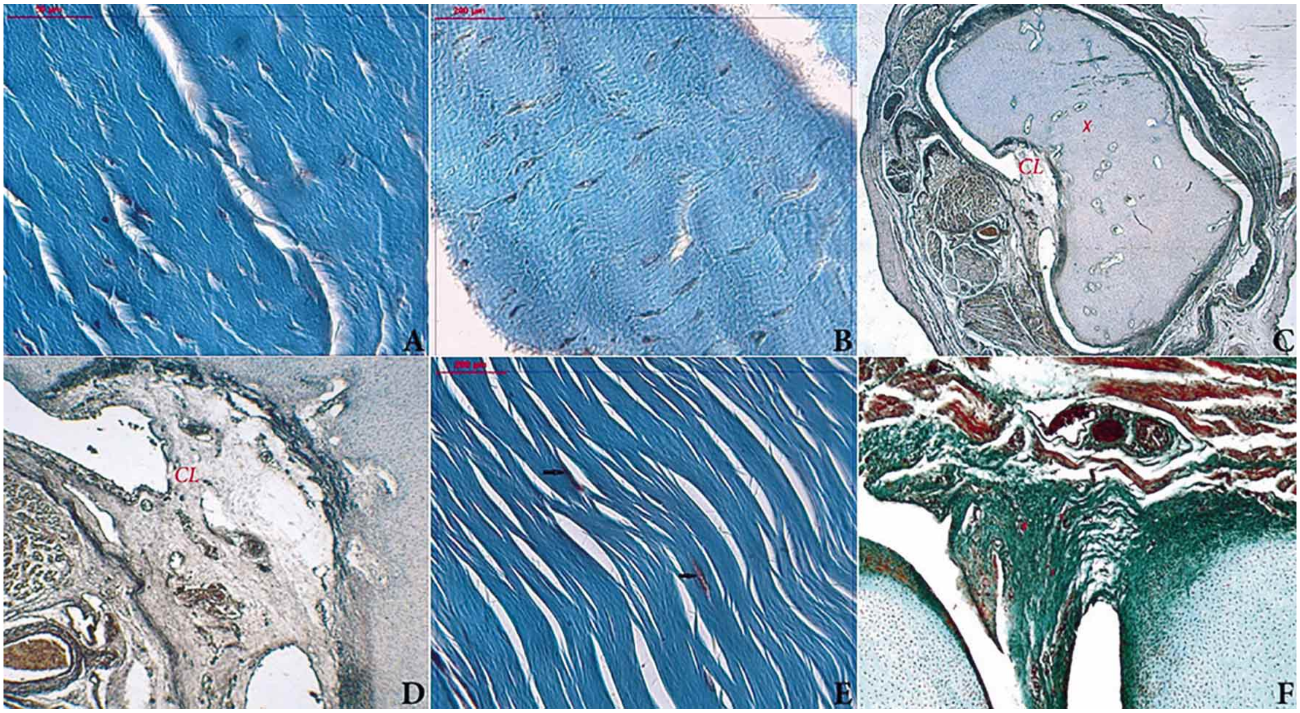

Fig. 3. Right knee anterior cruciate ligament of adult cadaver group. Lack of space between bundles of collagen fibrils and intensity is seen (Orcein-Picroindigocarmine staining, x40) (A). Left knee posterior cruciate ligament of adult cadaver group. Collagen fibril bundles and their proximity to each other are seen (Orcein-Picroindigocarmine staining, x40) (B). Right knee joint of 15-week-old fetus; articular cartilage (x), cruciate ligament (CL) (Orcein-Picroindigocarmine staining, x1.25) (C). Right knee joint of 15-week-old fetus; articular cartilage (x), cruciate ligament (CL) (Orcein-Picroindigocarmine staining, x10) (D). Tendon of right elbow triceps muscle of adult cadaver group; Collagen fibrils are seen parallel to each other while fibroblasts are monitored fairly long and narrow (Æ) (Orcein-Picroindigocarmine staining, x40) (E). Left elbow joint of 17-week-old fetus; collagen fibrils (co) forming radial annular ligament and fibroblasts (fb) which synthesize them. Collagens (co) are seen at lower concentration where the ligament attach to the bone (Masson-Trichrome staining, x10) (F).

distinguished clearly (Fig. 4B). In the image section of elbow joint in the coronal plane, while ulnar collateral ligament was isolated from surrounding structures in an obvious way, radial collateral ligament was observed to be mixed with existing surrounding tendons of muscle tissue (Fig. 4C, D).

As a result of examination of the knee and elbow joints ligaments belonging to seventeen-week fetus with 3 T MR, tibial collateral ligament, posterior cruciate ligament, radial collateral ligamentand ulnar collateral ligament were observed obvious (Fig. 4E, F). Despite, the ligaments were viewed significantly in magnetic resonance imagining of fetus with a 1.5 T MR unit,the fetal ligaments were not viewed clearly with 3 T MR.

DISCUSSION

Although, several studies regarding the development of knee and elbow joints have been performed, there are not many works available on the development of articular ligaments (Merida-Velasco *et al*.). In the literature review, we did not find any study examining the knee and elbow ligaments belonging to fetus and adults as a comparative with morphological, histological and MR images.

The main structures of ligaments are composed of collagens and fibroblasts which are synthesizing them. Some differences are also emerging during the parallel alignment of collagen fibers (Junqueira & Carneira; Merida-Velasco *et al*.; Amenta, 1983). In our study, the differences between the alingments of collagen, fibroblast density and morphological images of fetal and adult ligaments were observed.The most important of these was detected as fibroblasts and collagen density.

It was observed in the present study that, the ligaments of the lower limb of adult cadaver were thicker compared to upper limb ligaments. As a reason for this it was considered that upper limb is exposed to less pressure and stress than lower limb.

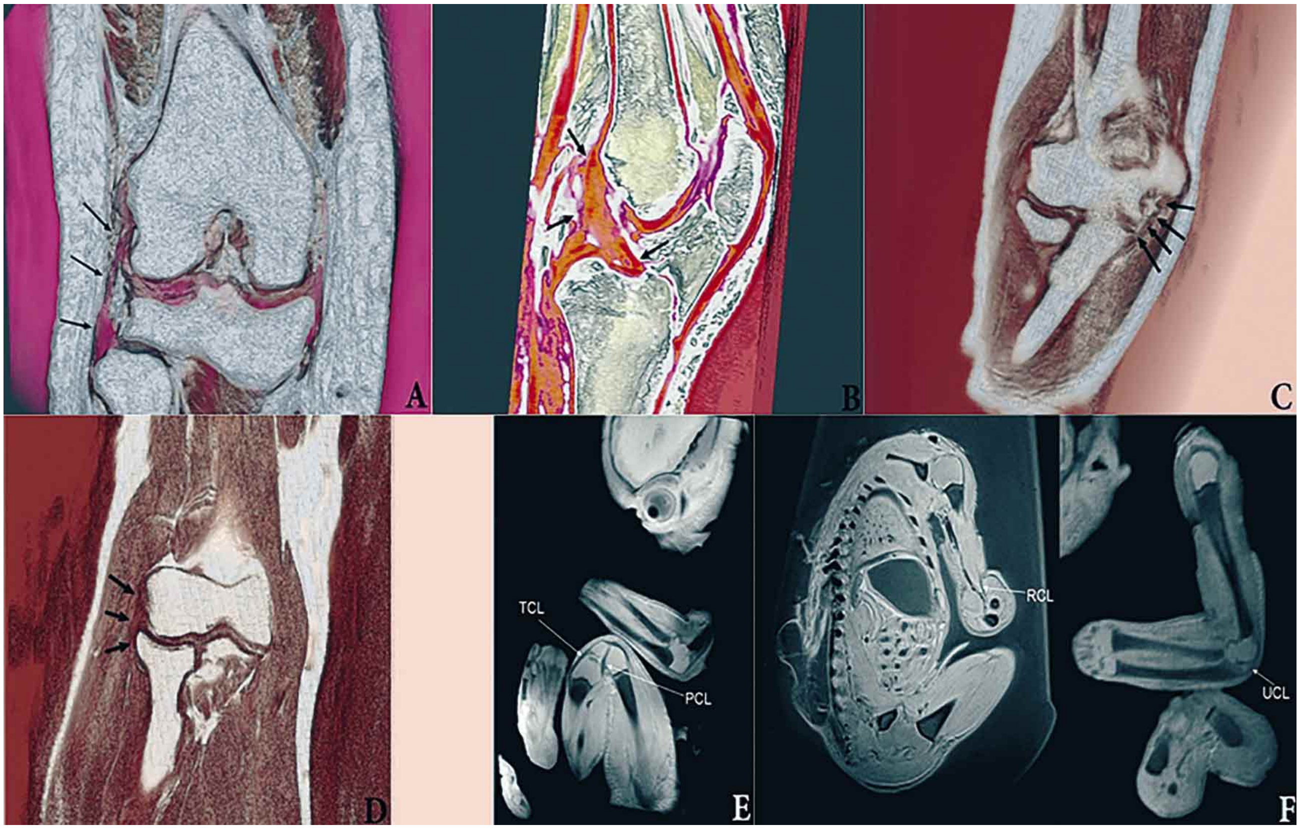

Fig. 4. Adult fibular collateral ligament (Æ). Three-dimensional MR in coronal plane of the knee joint (A).The three-dimensional of adult knee joint in the sagittal plane. Anterior cruciate ligament is seen (Æ) (B). Three-dimensional elbow joint of the ulnar collateral ligament (Æ) of adults, taken in coronal plane (C). Radial collaterale ligament (Æ) is seen on three-dimensional elbow of adults, taken in coronal plane (D). Tibial collateral ligament (TCL) and posterior cruciate ligament (PCL) is observed coronasagitally with 3 T (Tesla) MR s taken from 17 weeks old fetus (E). Radial collateral ligament (RCL) and the ulnar collateral ligament (UCL) is seen on 3 T (Tesla) MR s of 17-week-old fetus (F).

Some differences between adult knee joint ligaments were found. According to fibular collateral ligament, collagen fibers of patellar ligament and tibial collateral ligament were observed to be thicker. However, loose connective tissue from place to place was detected in the structures belonging to fibular collateral ligament. It is known that there is a correlation between the thickness of collagen fibers forming these ligaments and their strength countering the resistance, it is not a very strong correlation (Junqueira & Carneira).

The internal ligaments of the knee joint are quite stable (Williams *et al*., 1989; Snell). It was observed in this study, in stained sections of anterior and posterior cruciate ligaments that, collagen density were higher compared to other ligaments without a significant increase in the thickness of the collagen fibers. This suggests that, the density of collagen fibers affect the durability of the ligaments more than the thickness.

In a study conducted by Yahia and Drouin, it was detected that, although anterior cruciate ligament and patellar ligaments were similar structures, collagen structures in both structures had differences (Yahia & Drouin, 1989). In our study, it was detected that, the wavy course observed in the upper limb ligaments in adult group were not as significant as lower limb ligaments. Especially, this organization in the collagen fibers of the ligaments of the upper limb joint was considered to be caused by less stress on these joints and the number of movement is grater compared to the knee joint. These undulations were not very clear in the structure of the fetus.

It was detected in a study conducted by Meller *et al*. (2000) that, the fibroblast content of anterior and posterior cruciate ligaments in sheep, decreased from birth, whereas the amount of extracellular matrix showed an increase. Gardner & O´Rahilly (1968) in their study examining the human embryology and postnatal knee joint development, reported that, there was no change in cruciate ligaments unlike other ligaments. In our study, fibroblasts in fetal ligamentous structures were observed quite densely. Fibroblasts density in adult ligaments showed differences between ligaments. The amount of fibroblasts and collagen in both anterior and posterior cruciate ligaments of adult cadavers was observed extensively. In other ligaments that intensity was not detected.

The stains used to assess tendons and ligaments are also shared due to these two histological features shows were close properties to each other. In addition, animal studies on tendons and ligaments provide important information to us due to the structures belonging to laboratory animals and humans show similarity (Benjamin *et al*., 1991; Galatz *et al*., 2006; Kanazawa *et al*., 2006).

Amiel *et al*. (1984), revealed that, the fibroblast nuclei diameters belonging to tendons were thinner than those of ligaments. In the present study, the collagen structure and staining properties of the tendon belonging to triceps brachii that we have examined showed many similarities to the ligaments, fibroblasts were observed to be long and thin. The collagen structure of the tendons were similar to ligaments but there were some differences in terms of the morphology of fibroblasts.

Mahasen & Sadek (2000), detected that, anular ligament of radius had primarily flat shape and then started to be thicker. In the present study, we observed that, the number of collagen fibers, where anular ligament of radius adheres the bone, was lesser than the other parts. The observation that, radial collateral ligament does not give collagen fibers in the location to adhere the bone, made us believe that, the weakest part of the anular ligament of radii may be the closest part to the bone.

We find a chance to sufficiently evaluate the ligaments belonging to joints anatomically by using MRI. For the better assessment of the joints in MRI, all axial, coronal and sagittal images are needed (Brown & Semelka). The sections in our MR images were so thin as 0.7 mm, we were able to evaluate the ligaments in a very comfortable way. However, it was detected that, 1.5 T MR devices would only be sufficient in the assessment of the ligaments in adults, the assesment of ligaments of unborn fetus can be performed device with higher tesla. In this study, fetal knee and elbow joints ligaments were clearly evaluated with 3 T MR device. So, we think that, the use of 3 T MRin the assessment of the ligaments of unborn fetus will give more detailed information to clinicians compared to 1.5 T MR.

CONCLUSION

It is thought that the greater number and density of collagen fibers in adult ligaments when compared to fetal ligaments is linked to functionality.

The reason for the lower number and density of collagen fibers in the spot where the ligaments are linked to the bone in fetuses is due both to the fact that bone secondary ossification is not yet complete and the fact that joints and muscles in the spot have not yet fully developed and lack full functionality.

It has been established that fetal ligaments can only be seen in great detail with the use of very high definition (at least 3 T MR) MR's.

We are of the opinion that these findings will form a basis for further studies on muscular and skeletal systems.

ÖZEN, Ü.; ANIL, A.; ÖMEROGLU, S.; PEKER, T.; COSKUN, Z. K. & LIMAN, A. F. Investigación comparativa del ligamento conjunto de rodilla y codo con niveles estructurales en fetos y adultos. ***Int. J. Morphol., 33(4)***:1491-1501, 2015.

RESUMEN: El objetivo del estudio fue examinar y comparar los ligamentos de la articulación de la rodilla y del codo en fetos y adultos y determinar histológicamente como estos ligamentos cambian con el tiempo. Además, las imágenes de los ligamentos de las articulaciones fetales y adultas se examinaron con imágenes de resonancia magnética (IRM). Fueron utilizados 10 fetos masculinos entre 14 y 17,5 semanas, y dos cadáveres adultos masculinos obtenidos del Departamento de Anatomía, Facultad de Medicina de la Universidad Gazi junto con las IRM de las regiones de la rodilla y del codo de 10 hombres adultos obtenidos de Atatürk Educativa y del Hospital de Investigación entre los años 2009 y 2011. Para las secciones de rodilla y codo de los diez fetos y de los cadáveres masculinos se utilizó el mismo método de disección. Después de procesar los tejidos e incluirlos en parafina, se obtuvieron cortes en micrótomo los cuales fueron posteriormente teñidos con Tricrómico de Masson y Orceína-picro-índigo Carmín. Las secciones fueron fotografiadas y examinadas bajo microscopio. Se obtuvieron IRM del codo y de la rodilla de los fetos y adultos. Las diferencias encontradas entre los ligamentos de adultos y fetos estaban en relación a la densidad de fibroblastos y espesor de colágeno. Aunque no fue posible observar los ligamentos fetales con 1,5 Tesla (T) MR, se observaron claramente con 3 T MR. Las diferencias estructurales entre los ligamentos fetales y adultos se observan tanto a nivel histológico y de resonancia magnética.

PALABRAS CLAVE: Ligamentos de rodilla; Ligamentos de codo; Resonancia magnética; Fibras de colágeno; Fibroblasto.

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