A feasibility of ultrasonographic assessment for femoral trochlear depth and articular cartilage thickness in canine cadavers

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Abstract

Trochleoplasty or trochlear groove deepening is one of surgical correction techniques for patellar luxation (PL) that caused cartilage alterations of the distal femur and this technique may not be always necessary. To evaluate trochlear groove for PL correction preoperative planning, several diagnostic image techniques have been applied. In this study, ultrasonography (USG) was introduced in the trochlear evaluation as pre-operative planning because it's user-friendly and radiationless. USG imaging of 22 distal femurs from small breed of canine cadavers were evaluated for trochlear groove depth (TGD) and trochlear cartilage thicknesses (CT) at medial condyle (MCCT), femoral groove (FGCT) and lateral condyle (LCCT), and compared to those of other distal femoral evaluations such as conventional radiography and/or anatomical appearance observed through stereomicroscope (STR). The results showed that TGD on radiograph was significantly deeper than those on USG and STR (P = 0.0099 for USG and P = 0.0021 for STR) but TGD on USG and STR was not significant difference (P > 0.9999). MCCT, FGCT and LCCT were evaluated and compared only between USG and STR and the results showed that only FGCT between techniques were not significant difference (P = 0.0646). At the condyles, MCCT and LCCT on USG were significantly thicker than those on STR (P = 0.006 for MCCT and P = 0.0004 for LCCT). In conclusion, USG was a reliable technique for TGD and FGCT evaluations. However, to evaluate the MCCT and LCCT by USG, CT may be slightly exaggerating. This diagnostic imaging technique could be applied in clinical practice and further evaluations of bone appearance between the normal and PL dogs should be done.

Keywords: cartilage thickness, dog, femoral trochlea, stifle, ultrasonography

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Introduction

Patellar luxation (PL) is one of the most frequent orthopedic diseases found in small breed dogs in several countries (Vidoni et al., 2006; Soontornvipart et al., 2013). The etiology of this diseases is not only developmental disease, but can also be counted as an acquired orthopedic disease caused by trauma or orthopedics surgery (Hayes et al., 1994). Common features of PL include quadriceps displacement, shallowed femoral trochlear groove, torsion of tibial tuberosity and patellar alta (Hans et al., 2016). Furthermore, malposition of the quadriceps muscles can induce angulation and rotation between distal femur and proximal tibia. This phenomenon subsequently increases stress to articular cartilage and cranial cruciate ligament. Therefore, long term of stifle joint instability due to the progression of PL may lead to stifle osteoarthritis (OA).

In general, surgical correction by rearrangement of the quadriceps and stabilization of patella to be fitted in femoral trochlear groove is one of the treatments of choice (Kowaleski et al., 2012; van der Zee, 2015). Surgical corrections including both of soft tissue and bone reconstruction were reported to be recommended for PL in dogs. (Slocum, 1993; Hayes and Boudreiau et al., 1994; Arthers and SJ, 2006). Nevertheless, bone reconstruction with trochleoplasty; femoral trochlear deepening, was reported to cause the damage of hyaline cartilage and finally were permanently replaced by fibrocartilagenous tissue (Thompson, 1975; Hunziker, 2002; van der Zee, 2015). Fibrocartilage patch creation from trochleoplasty does not have the same properties as that of hyaline cartilage (O'Driscoll, 1998). In addition to fibrocartilageneous replacement following the trochleoplasty, the unfit between patella and new outline of the sulcus caused abnormal pressure at the stifle joint and led to cartilage degradation (Daems et al. 2009). Interestingly, Linney and colleagues (2011) reported that surgical treatment for grade II, III and IV of PL by only tibial tuberosity transposition (TTT) and lateral retinaculum imbrication in dogs without trochlear groove deepening showed satisfying results. These dogs showed only 19.8% of patellar reluxation and osteoarthritis were not found during eight weeks postoperatively. Therefore, to treat PL with surgical correction, trochlear groove deepening may not always be necessary, and preoperative planning by evaluating of distal femoral structures by means of trochlear depth and surrounding cartilage condition should be done prior trochleoplasty selection.

To achieve the evaluation of distal femoral structures, several imaging modalities such as conventional radiography and computed tomography have been reported. On radiograph, noticeable transformation of the joint could not be promptly observed in early osteoarthritis (Innes et al., 2004; Marino and Loughin, 2010; Alam et al., 2011). In contrast, advanced technique such as computed tomography images of measurement can provided the three-sided depths of the trochlear groove; proximal aspect gauged at the level of the proximal point of the fabellae, distal aspect gauged at proximal to the intercondylar notch and the center aspect gauged halfway between the distal and proximal points (Towle et al., 2005). Although,

computed tomography can provide three-dimensional information, several issues should be concerned. For example; high radiation exposure comparing to radiography, requirement of chemical immobilization such as deep sedation or anesthesia and also requirement of strict positioning (Marino and Loughin, 2010).

In addition to radiography and computed tomography, ultrasonography (USG) that is a low cost, non-invasive and radiation less, is comfortably technique for animals. Althogh the skyline view of the stifle, which the patellar is vertically direction to the Xray beam from cranioproximal to craniodistal direction (Meier et al., 2001), is a routinely procedure to observe distal femoral trochlear. This study selected the gross appearance of bone structure on STR as the gold standard in stead of skyline view due to stifle radiograph could not provide information of the trochlear cartilage (Wolski et al., 2011). USG can be done in conscious dogs or under only light sedation. Besides, USG can be useful for clinical evaluation of almost all intra-articular structures such as articular cartilage, intra-articular effusion and articular tendon (Marino and Loughin, 2010). Laasanen and colleagues (2006) reported that USG could be applied as quantitative assessment of the impaired structural integrity of cartilage and subchondral bone in porcine model. In dogs, it has been reported that USG was used to estimate structure of femoral joint (Kramer et al., 1997; Hansen et al., 2017). Since the PL is one of the most frequent orthopedic diseases found in small dogs and USG is one of the imaging modalities that current broadly available, USG observing the trochlear depth and adjacent structures at the stifle would be one of the attractive procedures for preoperative surgical planning for PL. Therefore, the aim of this study was to assess distal femoral structures, all of trochlear depth and articular cartilage thicknesses (CT) at the distal femur, through USG comparing to the stifle radiographs and gross anatomy in canine cadavers.

Materials and Methods

Animals: Twenty two stifle joints acquired from 11 small breed dogs after death at the surgical unit of Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University between April 2018 and October 2019 under permission of owners. All cadavers were mature without history of stifle fracture or luxation, metabolic disease, and stifle surgery. If stifle deformity was detected and there were history of PL, the cadaver will be excluded from the study.

Prior the study procedure, all samples were marked at the deepest level of the trochlear groove using two Kirschner wires (K-wire) before studied as a marker of each techniques (Fig. 1). To achieve the location, skin at the lateral and medial sites of distal femur were stabbed to locate the long digital extensor tendon. Guided pins were then parallel drilled parallel drilled at the cranial and caudal to origin of the long digital extensor tendon from the lateral site to the medial site by using a battery power drill. The distance between two pins marks was 3 mm. The drilling procedure was slowly done to ensuring that pins did not bend and interrupt when flex and extend of stifles

during observation. K-wires (0.1 mm in diameter) were then replaced in the guided holes.

Radiography of the distal femur: All radiographs of the stifle joints were done with sternal recumbency and the pelvic limbs were maximal flexion (Towle *et al.*, 2005). The primary X-ray beam was vertically centered to the femoral trochlear groove. Calibrate ball was placed at the level of femoral trochlear for calibration of all radiographic parameters. As soon as the best

positioning was achieved, skyline stifle radiograph (X-ray) was taken. The trochlear groove depth (TGD) was measured at the most depth of groove comparing to adjacent condyles (Fig. 2A and 2B). TGD was done by measuring in two directions; horizontal and vertical lines. At first, the horizontal line was drawn from the highest point from medial to lateral trochlea and vertical line was then measured at the most depth of the trochlear groove until the horizontal line and reported as TGD (Fig. 2A).

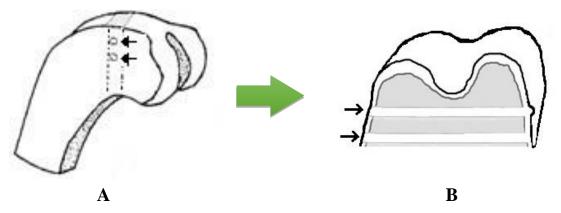


Figure 1 The anatomical marks at the distal femur before the experiment to validate the similar location among imaging procedures. A: a lateral view of distal femur and B: the cross section view of femoral trochlea site.

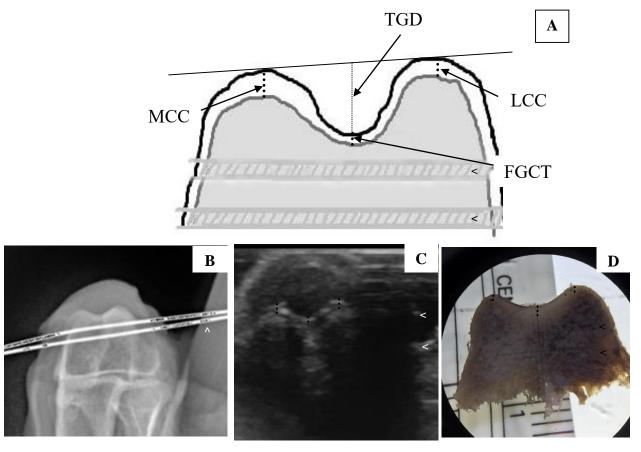


Figure 2 Distal femoral trochlear on different imaging procedures. A: an illustration of distal femur represented the measurement methods for trochlear groove depth (TGD), all dashed lines were presented cartilage thickness (CT) including of medial femoral condyle cartilage thickness (MCCT), trochlear groove cartilage thickness (FGCT) and lateral femoral condyle cartilage thickness (LCCT); B: a skyline stifle radiograph; C: the transverse ultrasonographic image (USG) of distal femur; D: the transverse view of distal femur observed through stereomicroscopic image (STR). Pins sites were presented with arrow heads.

Ultrasonography of distal femur: All stifles were examined by USG scanner (LOGIQ P6®, GE, Seoul, Korea) using a 10 MHz, linear transducer on transverse view at maximum flexion of the stifle. The USG beam was vertically centered over the trochlea. After the best view of femoral trochlear with the evidence of double K-wires was detected on the cine loop, USG image was captured, Subsequently, all parameters such as TGD and adjacent cartilage thichnesses; medial condyle cartilage thickness (MCCT), femoral groove cartilage thickness (FGCT) and lateral condyle cartilage thickness (LCCT) were measured using digital caliper (Fig. 2A and 2C). To measure the CT, only the thicknesses of hypoechoic line at the highest point of medial and lateral condyles, and trochlear groove above the hyperechoic bone cortex were collected (Fig.

Stereomicroscopic examination for distal femur: All parameters such as femoral trochlear depth and various sites of cartilage thickesses of distal trochlear were grossly observed by stereomicroscope (STR) as the gold standard and compared all parameters to those of the radiograph and USG images. As soon as the radiographic and USG examinations were done, both K-wire were removed. Then, distal femurs were cut using oscillating saw (Synthes®, Johnson and Johnson, Germany) for anatomical observation using stereomicroscope (STR). Bones was cut at the K-wire marking sites with the 0.5 mm of sample thickness. Subsequently, bone samples were reserved in a 5% formaldehyde solution before observing of TGD, MCCT, FGCT, LCCT as USG technique by STR (Olympus®, Olympus Corporation, Tokyo, Japan) within twenty-four hours (Fig. 2A and 2D).

Statistical analysis: All clinical demographic data including image parameters consisting of TGD, MCCT, FGCT and LCCT were presented as means with standard deviations. All data were tested using GraphPad Prism software version 8.2.0 (GraphPad Software, California, USA). At first, the normality test was done by the Shapiro-Wilk test. To compare the TGD among method, Friedmann test was applied. In

addition, to compare the significant difference of two data set such as parameters between left and right limb or parameter between USG and STR, a pair t-test and Wilcoxon test were used for normal distributed data and non-parametric data set respectively. Besides, the correlation between the CT and body weight was test using linear regression. All statistical analyses were significant if the P value < 0.05.

Results

Clinical demographic data: Twenty-two stifles samples were collected from eleven canine cadavers. There were pomeranian (n= 4), shih-tzu (n=3), and one of each following breed: chihuahua, Lhasa apso, miniature Pinscher and mongrel. There were six male and five female cadavers. The mean body weight was $4.94 \pm 1.49 \text{ kg}$ (2.5 - 7.0 kg). The mean age was $11.09 \pm 2.91 \text{ years}$ (5 - 13 years).

The trochlear groove depth (TGD): The TGD among investigation techniques were reported as mean \pm SD, 95% confidence interval and range (Table. 1) FDG on the skyline stifle radiograph was significantly deeper than those on the USG (P = 0.0099) and STR (P = 0.0021) whereas TGD on the USG and STR were not significantly difference (P > 0.9999).

The femoral trochlear cartilage thickness (CT): CT of different areas at the femoral trochlear were collected and compared only on USG and STR (Table. 2). CT at the FGCT was comparable between USG and STR (P = 0.0646). In contrast, the CT at lateral sites of the image, both of MCCT and LCCT were significant difference. The MCCT and LCCT on the USG were significantly thicker than those of those on the STR (P = 0.006 for MCCT and P = 0.0004 for LCCT, respectively; (Fig. 4).

Relationship of femoral groove CT and body weight: Considering with body weight of canine cadavers, the FGCT seemed to be correlated with the body weight, however, statistical significance differences were not detected on both of USG and STR (Fig. 5).

Table 1 The femoral groove depth (FGD) among observation technique; skyline stifle radiograph (Xray), ultrasonographic image (USG) and stereomicroscope (STR)

Imaging modality	Mean ± SD	95% Confidence interval	Range
Xray	1.31 ± 0.16	0.341043	0.28 - 3.45 ^{a,b}
USG	0.83 ± 0.07	0.154087	0.29 - 1.6a
STR	0.78 ± 0.11	0.237899	0.20 - 2.38 ^b

^a; P = 0.0099

Table 2 The femoral trochlear cartilage thicknesses obtained by ultrasound image (USG) and stereomicroscope (STR)

Imaging modality	Mean ± SD	95% Confidence interval	Range
USG	0.45 ± 0.04	0.07381	0.28 - 0.90a
STR	0.42 ± 0.04	0.07487	$0.25 - 1.00^{a}$
T 0.07			

 $^{^{}a}$; P = 0.06

b; P = 0.0021

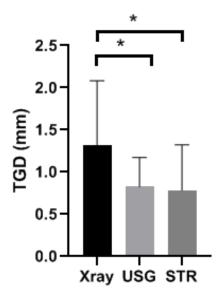


Figure 3 Trochlear groove depth (TGD) on skyline stifle radiography, ultrasonographic image (USG) and stereomicroscopic image (STR). The trochlear groove depth (TGD) on radiograph was significantly deeper than those of the USG (P = 0.0099) and STR (P = 0.0021). The trochlear groove depth (TGD) on between ultrasonographic image and stereomicroscopic image were not significantly significant difference (P > 0.9999).

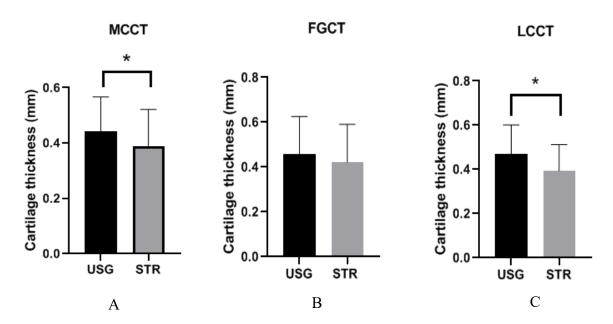


Figure 4 Femoral trochlear cartilage thicknesses (CT) at medial femoral condyle cartilage thickness (MCCT) (A), trochlear groove cartilage thickness (FGCT) (B) and lateral femoral condyle cartilage thickness (LCCT) (C) on ultrasonographic image (USG) and stereomicroscopic image (STR). There was no significantly difference between two methods (P = 0.0646). However, There were significantly difference between MCCT ($P^0 = 0.006$) and LCCT ($P^0 = 0.0004$).

Discussion

Despite radiography is the most common imaging modality for musculoskeletal diagnosis in dogs (Kramer *et al.*, 1997), USG was an interesting modality that was applied to investigate the intra-articular structures of stifle joint due to the scanner availability and radiation less technique compared to those of other modalities (*Karim et al.*, 2004; *Soler et al.*, 2007). USG can provide the information of femoral trochlear appearance. Besides, trochlear cartilage can be observed in human stifle (Kazam *et al.*, 2011). In addition to human study, there was a study of using of

USG for stifle in small animals such as fox. However, the USG results compared to those of radiograph and gross anatomy of distal femoral trochlear without calibrating marker may affect the accuracy of the results (Miles *et al.*, 2014).

In this study, canine cadaver was selected to be the sample of the study instead of alive canine patients due to the availability of the femoral trochlea for gross measurement without any invasion. All selected cadavers was donated and processed for the study soon right after death or within 24 hours with preserving the cadavers at 0-4 degree Celsius. Previous

study reported that human chondrocytes remained viable at post mortem period (Alibegović *et al.*, 2014). The viability of chondrocytes was not significantly different at 9 days at post-mortem when keeping the sample at below than 35 degree Celcius (Alibegović *et al.*, 2014). Therefore, the period and condition of stifle in this study could not effect to the quality and quantity of femoral trochlear articular cartilage.

Before the study, all distal femurs of canine cadavers were marked with two parallel pins to confirm the comparable location among measurement methods. The location to insert marking pins was selected to be the deepest point of the femoral trochlear or at the insertion of the long digital extensor tendon.

All parameters such as femoral trochlear depth and various sites of cartilage thickesses of distal trochlear were grossly observed by STR as the gold standard and compared all parameters to those of the radiograph and USG images. In this study, histological sample of distal femoral trochlear under the microscope did not prefer for evaluation since—the routine chemical fixation solution for bone and cartilage such as aldehyde based medium can create the disruption of the chondrocyte surfaces and caused the condrocyte retraction and shrinkage (Hunziker, 2002). Therefore, observing of CT of distal femoral trochlear on histologic samples comparing to those on USG images or gross appearance might not be accurate.

FGCT on STR FGCT on USG Cartilage thickness (mm) Cartilage thickness (mm) 0.8 r=0.31. P=0.0766r = 0.28. P 1.0 0.6 0.5 0.0 2 6 8 0 4 2 6 0 4 8 body weight (kg) body weight

Figure 5 A correlation between body weight and femoral groove cartilage thickness (FGCT) on ultrasound image (USG) and stereomicroscopic image (STR).

Besides, to reach the hyperflexion of the stifle for skyline view by the sternal recumbency observation for appearance and depth of trochlear would be interfered by the volume of the tight muscle. The more hyperflexion of the thinner tight muscle could be more achieved than that of the thicker tight muscle. Therefore, on the sternal recumbency, hyperflexion of stifle in different muscle volumes of the tight would effect to the angle of stifle and location (Miles *et al.*, 2014) of distal trochlea that need to be perpendicular to the primary x-ray beam. The different location of the trochlear depth in different dog would be effect. Such that, the radiographic assessment is inconclusive and further advanced imaging techniques are necessary (Marino and Loughin, 2010).

To observed on the STR, distal femur was cut at the same direction of the double marking pin. At first, the trochlear groove depths among modalities of radiograph, USG and STR were compared. The trochlear groove depth on radiograph showed the deepest value compared to those on USG and STR. The deepest of the trochlear groove on radiograph may be due to radiograph provided information of only the cortical bone without the CT (Towle *et al.*, 2005; Hansen *et al.*, 2017).

In addition to trochelar groove, in this study, we investigated the differences of femoral trochlear

cartilage thickess only USG and STR. The result suggested that the cartilage thickess only at trochlear groove was comparable between methods of USG and STR but not the condylar cartilage. To perform USG in this study, linear transducer that produced linear array and image formation showed as rectangular image format was used (Von Ramm and Smith, 1983). Linear transducer was focusing of the ultrasound beam that can be achieved by a concave lens with only in one direction (Aldrich, 2007) Ultrasound beam reflection firstly arrived at the center of the array. A few microseconds later, the ultrasound beam was reflected at the outer edges. In according to the delay of the ultrasound reflection at the side or the border of the image, the less tightly focused beam of ultrasound can be generated (Powers and Kremkau, 2011). Therefore, the difference of CT at the medial and lateral condyles observed between the USG and STR could be caused by the location of the structure on ultrasonogram. The more centralized location is preferred to enhance the accuracy of the observation. In addition to the location of the structure on ultrasonogram, frequency or sounds speed seems to impact on the CT too. In the difference of the structure such as tissue type, species and pathology of the tissue could effect on the sound speed (Myers et al., 1995). To clarify the effect of sound on the cartilage among differences of sample such as breed

and body weight including the variation of bone pathology and the evaluation of enhancing sound transmission instrument (Shen *et al.*, 2014), further investigation should be done.

In addition to differences of trochlear structure among imaging modalities, the CT seemed to have a correlation to the bodyweight of cadavers. However, due to the small number of the sample size in this study, statistical significance was not detected. Therefore, further study with increasing number of the population of dogs would provide more information. Moreover, this study was not compare the feasibility of the pre-operative USG to the gross appearance of femoral trochlear of patella luxation patient by means of the sensitivity and specificity of USG to detect the cartilage lesion. Therefore, further study should be done.

In conclusion, the USG provides more precise information of the distal femur such as trochlear groove depth and CT than that of the stifle radiograph. Although, the location and direction to evaluate the CT of distal femoral trochlear by USG must be performed when the sound beam is only centralized to the interested anatomical structure, this technique can be clinically applied in veterinary practice both of preoperative diagnosis and proper treatment planning for patella luxation and also postoperative osteoarthritis detection.

The limitation of this study was that included samples in this present study were senior dogs. In addition, postmortem changes, as well as, the preparing cadaver procedure (freezing, thrawing arthrotomy) may influence on cartilage properties. Therefore, it should be further investigated in living dog. Moreover, increase number of the samples of dogs might be provide relationship between demographic data and visual information for each imaging modalities. Moreover, Ultrasound beam reflection firstly arrived at the center of the array. A few microseconds later, the ultrasound beam was reflected at the outer edges. In according to the delay of the ultrasound reflection at the side or the border of the image, the less tightly focused beam of ultrasound can be generated (Powers and Kremkau, 2011).

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