The torsional alignment changed significantly of hindlimb in growing rabbits after patellar dislocation

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Abstract

Background Torsional malalignment has been considered as a risk factor for patellar dislocation. But the influence of patellar dislocation for torsional alignment development remains unknown. The present study aims to investigate whether the torsion alteration of the hindlimb occur after patellar dislocation in growing rabbits. Methods In the present study, 30 one-month old rabbits were included. The experimental group consists of 30 left knees of rabbits and were underwent patellar lateral dislocation. And the control group consists of 30 right knees and no surgical procedure was performed. The Computed Tomography (CT) scan was performed on each knee when the surgery was finished and at the time the rabbits were skeletal mature (5 months post-surgery). The angles of femoral version and tibial torsion were measured using a three-dimensional method and analyzed between the experimental group and control group. Results The femoral version and tibia torsion in the experimental and control group immediately after surgery were not significantly different. However, 5 months after surgery, the femoral version angle of the experimental group (-5.50±6.13°) was significantly different with that of the experimental group (-10.90±4.74°)( P < 0.05). But the tibia torsion angle in the experimental group (7.17±7.25°) and control group (4.47±6.34°) were not significantly different ( P = 0.144). Conclusion From this study, patellar dislocation can lead to alteration of femoral version in growing rabbits. This may indicate the early treatment for patellar dislocation in children is particularly important to avoid torsional malalignment in the future. These findings may develop pathology and etiology of patellar dislocation.

Background

Several anatomic factors are associated with patellar dislocation, including increased tibial tubercle–trochlear groove (TT-TG) distance, patella alta, rotational deformities, trochlear dysplasia and patella shape [1, 2, 3, 4, 5, 6]. Among the factors, rotational malalignment has been regarded as a risk factor for patellar dislocation in previous studies[2, 3, 4, 5, 6]. For femoral anteversion, Dejour et al. [2] found that the average femoral anteversion of normal knees was 10.8°, but the average femoral anteversion of knees with patellar instability was 15.6° (P = 0.013). Erkocak[3] and Takagi[4] also found the patients with a history of patellar instability have a higher mean femoral anteversion. For tibia torsion, no significant difference were found between patients with patellofemoral instability and the normal[3, 5, 6]. Clinically, strategic choice of surgical treatment for patients with patellar instability may be influenced by the torsion of the lower limbs. For patients with patellar instability who have a femoral anteversion higher than 25°, patellofemoral ligament reconstruction for patellar instability only may be insufficient and derotational femoral osteotomy should be considered[7].

Previous studies have investigated the influence of patellar dislocation on the morphological development of the patellofemoral joint. Wang, Li and Niu observed femoral trochlear dysplasia and patellar dysplasia could occur after patellar dislocation in growing rabbits[8, 9, 10]. Kaymaz found trochlea flattening after surgery for creating patella alta in growing rabbits[11]. These studies indicated that patellofemoral joint dysplasia could be caused by patellar instability. In the study by Niu[12, 13], tibial tubercle lateralization and tibial tuberosity–trochlear groove distance (TT-TG) increased after patellar
dislocation. TT-TG reflects the relative location between distal femur and proximal tibia, and reflects the lower extremity alignment. So it is possible that patellar dislocation have influence on lower extremity alignment directly.

Although low extremity malalignment is considered as a predisposing factor for patellar dislocation[2, 3, 4], the effect of patellar dislocation on low extremity alignment development has remained unclear. Based on the previous studies, we hypothesized that early patellar dislocation lead to a significant difference of alignment of hindlimb in growing rabbits. The objectives of the present study were to elucidate the alignment alteration in transverse plane after patellar dislocation in growing rabbits and discuss the influence of patellar dislocation on lower extremity alignment.

**Materials And Method**

**Study Design and Setting**

This study was approved by the Animal Ethics Committee of the third hospital of Hebei Medical University (Number: Z2019-006-1). Sixty knees from 30 healthy, 1-month-old female New Zealand white rabbits, weighing between 350 and 450g (provided by the Animal Center of the Hebei Medical university), were split into two groups. The experimental group consisted of 30 left knees, which were performed patellar dislocation surgery. The control group comprised the 30 right knees, and no surgical procedure were conducted. All procedures performed in studies involving animals were in accordance with the Western University's Animal Care and Use Guidelines.

The rabbits were kept individually in cages (310 × 550 × 320 mm), under controlled temperature (22 ± 2°C), humidity (55 ± 5%), 12-hour light-dark cycle (7:00 a.m. to 7:00 p.m.). The rabbits were raised with unrestricted access to standard water and food and were allowed 30 minutes activity out of cages per day. The rabbits were euthanized by excessive anesthesia of pentobarbital sodium by injection through air vein at the end of study.

**Surgical Procedures**

The processes for making patellar dislocation models of growing rabbits have been described and proved by previous studies[8, 9, 10]. The one-month old rabbits were given anaesthesia of ketamine hydrochloride and xylazine at a dosage of 20 and 5 mg/kg body weight by injection though ear vein. The rabbits were fixed on the platform for surgery with spine position. Then, the left knees of the experimental group were shaved and disinfected by standard procedures. A 2.5-cm incision was performed on the middle line of the knee skin, and the medial retinaculum and the joint capsule were exposed. The medial retinaculum and medial joint capsule of the knees were incised about 1.5-cm and the patella was then pushed laterally with haemostatic forcep to expose the femoral trochlea. Then the lateral joint capsule was sutured with overlapping tissue. After these procedures, patellar dislocation could be seen
intraoperatively (Fig. 1). The patella dislocated (the femoral trochlea was exposed) when the knee was flexed and extended. All the procedures were performed carefully to avoid cartilage and blood vessel damage. Then the incision was sutured, and bandages were applied over the incision. CT scans were performed immediately after surgery to confirm lateral patellar dislocation. Ciprofloxacin (10 mg/kg, po) was administered three days postoperatively for prophylaxis. As we all known, the growing rabbits achieve skeletal maturation at 6 months, so both groups were followed-up for 5 months after surgery (The rabbits were 6 months old)[13]. After that, the rabbits were euthanized by excessive anesthesia of pentobarbital sodium by injection through air vein.

CT assessment

CT scans of the rabbits were performed immediately after operation and 6 months post-operatively using a 16-slice CT scanner (SOMATOM Sensation 16; Siemens Medical Solutions, Erlangen, Germany). The rabbits were under anaesthesia and were placed in a supine position. The hindlimb were fully extended and were fixed on a board to prevent any movements during scanning. Contiguous slices (1.0 mm) were obtained from the upper rim of the pelvis to the most distal part of the hindlimb. Considering the different structure of the hindlimb in rabbits and the accuracy of the measurements, the measurements were performed in a 3-dimensional strategy just as the previous studies showed [4, 14, 15, 16, 17, 18]. The CT slices were sent to RadiAnt DICOM software (Medixant Ltd., Poznan, Poland) and reconstructed for 3D models. Our measuring methods had an accuracy of 0.01°.

The femur version were measured as the study by Jia showed [14]. After 3D image construction, the femur was given a superior view (Fig. 2a). The lowest point of the greater trochanter, the femoral medial condyle and femoral lateral condyle were moved and rotated for adjustment until the lowest point of the greater trochanter were positioned in the middle between medial and lateral femoral condyle. The three points were connected by the horizontal line B. The femoral neck version was the angle formed by the line B and the line A which connecting the point of the femoral head center with the midpoint of the narrowest femoral neck part (Fig. 2b). The condition that femoral neck is anterior to posterior condylar line is defined positive.

For tibia torsion measurement, as the previous study [4], the tibia was given a inferior view after 3D image reconstruction. (Fig. 3a). The tibia was rotated and moved until the tibia shaft was almost covered by ankle joint. The most posterior points of the medial and lateral tibia condyle were connected by Line D. Tibia torsion was measured by the angle between the line D and the line C which was drawn through the center of lateral and medial malleoli (Fig. 3b). The condition that the ankle laterally rotated to the posterior tibial plateau is defined positive.

Statistics
Statistical analysis was performed using the SPSS version 21.0 (SPSS, IL, USA). The results are expressed as mean ± standard deviation. The Levene's test were used to evaluate the homogeneity of the data. The mean difference of femoral version and tibia torsion between the control group and the experimental group were evaluated by Student's *t* test. A *P* value < 0.05 was determined as statistically significant.

For determining the intra-observer variation, the observer One repeated the observations 2 weeks after first measurement. To determine the inter-observer variation, the observations were performed by observer One, observer Two and observer Three. Intra-observer consistency and inter-observer consistency were analysed using intra-class correlation coefficient (ICC). ICC higher than 0.75 was regarded as excellent, and ICC lower than 0.40 was poor, ICC among 0.40 to 0.75 was fair to good.

From previous study [4, 19], the femoral version was selected as the primary variable for sample size calculation, the difference was set as 5° between the two groups, the standard deviation was assumed as 6°. With a confidence level of 95% (α = 0.05) and power (1-β) of 80%, the power calculation was performed and 24 knees were needed per group.

### Results

From CT scans, all the patellas dislocated laterally from femoral trochlea, which showed patella dislocation model were achieved successfully. In this study, 30 rabbits were measured the femoral version and tibia torsion immediately after surgery, and the values were not significantly different between the experimental group and the control group (Table 1). Two rabbits died of postoperative infection in one week after surgery. So 28 rabbits were taken CT scanning 5 months after surgery. At that time, the femoral version of the experimental group (-5.50 ± 6.13°) was significantly different with that of the experimental group (-10.90 ± 4.74°). But the tibia torsion in the experimental group (7.17 ± 7.25°) and control group (4.47 ± 6.34°) were not significantly different (Table 2). The intra-observer consistency and interobserver consistency were showed in Table 3.

### Discussion

The most important finding of this study is that femoral version is significantly different between study group and control group in growing rabbits after patellar dislocation, while the tibia torsion was not significantly different between two groups.

Femoral version shows the relative position of the femoral neck and the transcondylar axis or coronal plane of the distal femur. Femoral anteversion refers to anterior rotation of the femoral head from the coronal plane of the femur. While femoral retroversion is defined as the condition that the femoral neck axis locates posterior to the transcondylar axis or the coronal plane of the femur [20].

For human beings, the femoral anteversion at birth is 30° to 40°, then it decreases to 10° to 15° when skeletally mature. Most of the alteration occurs before the age of 8 years[21, 22]. For rabbits, there were
10° of anteversion in the femur at first. The anteversion disappeared by the eighth week and by the time the rabbits was skeletally mature, 10° to 15° degrees of femoral retroversion has been observed[19]. Although the decreasing trends of the femoral version development between human-beings and rabbits may look numerically similar, the femoral version between them was different. Actually, for adults, femoral retroversion is not as common as femoral anteversion. In the study by Hartel, a total of 1070 thin-slice CT scans of left femurs were analyzed and 77 subjects (7.8%) were found with retroverted femur (range -23.6° – 0.2°)[23].

Femoral version relates to the stability and function of the knee and hip joints and abnormal femoral version affects many disease, including torsional syndromes, fractures of femur, hip dysplasia, Legg-Calve-Perthes disease and anterior cruciate ligament (ACL) rupture[24, 25, 26, 27, 28]. Femoral version also affects patellar stability. The increased femoral anteversion has been regarded as a risk factor for patellar instability, as it produces a lateralizing force on the patella[29]. The lateralizing force exists even after medial patellofemoral ligament reconstruction, contributes to the inferior clinical outcomes, even reconstruction failure[30, 31].

In this study, the femoral retroversion decreased after patellar dislocation in growing rabbits. Patellar dislocation may cause the alteration of the strength direction of rectus fomoris muscle. Also, we found knee or ankle lateral rotation in activities of rabbits after patellar dislocation. The alteration of strength direction and bone position may be the reason for the femoral version difference during growth. The version of the femur changed significantly after patellar dislocation, but the tibia torsion did not change significantly in the growing rabbits after patellar dislocation. Similar with humans, in the lower extremity, the femur may be abnormal obviously but the tibia and fibula are well formed or only slightly hypoplastic. And the foot may be normal despite the severe anomalies in the proximal part of the lower extremity[32].

For the previous animal experiments[8, 9, 10, 12], patellar dislocation or instability could led to femoral dysplasia, patella dysplasia and higher TT-TG. These factors were also regarded as risk factors for patellar dislocation[1, 2, 3, 4, 5]. So femoral dysplasia, patella dysplasia and high TT-TG are not only risk factor for patellar dislocation, but also could be the consequences of patellar dislocation. In the present study, the aberrant femur version was observed after patellar dislocation in the growing rabbits. Similarly, abnormal femur version may not only be a risk factor for patellar dislocation, but also be the consequence of patellar dislocation. These findings may develop pathology and etiology of patella instability, and emphasize the importance of the early effective treatments for patella instability in children, considering the possibility of pathological conditions caused by femur version deformity.

From previous studies, patellar dislocation has been successfully achieved in growing rabbits after patellar dislocation surgery[8, 9, 10, 12]. In the present studies, patellar dislocation was observed by CT scans from each rabbit immediately after surgery and at the last follow-up, which showed the patellar dislocation model was obtained successfully. It is possible that alignment values have not been measured precisely because two-dimensional (2D) measurements can be affected by the location of the radiation source and the limb position[4]. Recently, three-dimensional (3D) method for measuring the
alignment of lower extremity has been widely used, which was proved to have high intra-observer and inter-observer reliability. And the method is not influenced by femoral neck-shaft angle or postural deformity\[4, 14, 15, 16, 17\]. Considering the high accuracy of the method and the extreme flexion of knee and hip joints in rabbits, the 3D method has been taken account in this study and achieved high intra-observer and inter-observer reliability (Table. 3).

There are several limitations of the study. First, the structure of the hind limbs of rabbits are different from human beings'. For example, adults often have femoral anteversion while mature rabbits often have femoral retroversion. So the conclusion of this study may not be applied for humans. But the rabbit models have been widely used for the patellar dislocation studies\[8, 9, 10, 12\]. The present study is the first research focusing the influence of patellar dislocation to torsional alignment and has high intra-observer and inter-observer reliability, which may enrich the etiology and pathology of patellar dislocation. Second the knee rotation measurements were not involved in this study because of the extreme flexion in the knee joints in the rabbits. But the femoral version and tibia torsion can sufficiently reflect the torsional alignment of rabbit hindlimb. The third limitation is the sample size of the rabbits. And it could be more reliable if a higher number of experimental animals were used. But the sample size is enough according to the sample size calculation. Also, the reason for the alteration of femoral version in biomechanical and molecular level should be researched in the future.

**Conclusion**

Based on the outcomes of this study, we conclude that early patellar dislocation can lead to abnormal femur version in growing rabbits. So patellar dislocation may have an effect on lower extremity alignment. Clinically, early intervention for adolescent patients with patellar dislocation will be particularly important to avoid the deformities of patellofemoral joint and lower extremity alignment.

**Declarations**

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**Authors’ contributions**

FW designed the study. JHN, QQ, and KH performed the experimental work.

JHN, QQ, and KP evaluated the data. JHN wrote the manuscript. All authors read and approved the final manuscript.
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Availability of data and materials

The detailed data and materials of this study are available from the corresponding author via e-mail on reasonable request.

Ethics approval and consent to participate

Institutional review board approval of the Animal Ethics Committee of the third hospital of Hebei Medical University (Number:Z2019-006-1) was obtained.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Measurements immediately after operation

| Measurement     | Experimental group | Control group | N  | P value |
|-----------------|--------------------|---------------|----|---------|
| Femoral version | 11.88 ± 4.89       | 13.50 ± 5.51  | 30 | 0.205   |
| Tibia torsion   | 11.56 ± 4.03       | 12.94 ± 3.48  | 30 | 0.164   |

Significantly different: $P < 0.05$. Femoral version: positive: femoral neck is anterior to posterior condylar line; negative: femoral neck is posterior to posterior condylar line. Tibia torsion: positive: ankle laterally rotated to the posterior tibial plateau; negative: ankle internally rotated to the posterior tibial plateau.

Table 2. Measurements five months after operation

| Measurement     | Experimental group | Control group | N  | P value |
|-----------------|--------------------|---------------|----|---------|
| Femoral version | -5.50 ± 6.13       | -10.90 ± 4.74 | 28 | 0.001   |
| Tibia torsion   | 7.17 ± 7.25        | 4.47 ± 6.34   | 28 | 0.144   |

Significantly different: $P < 0.05$. Femoral version: positive: femoral neck is anterior to posterior condylar line; negative: femoral neck is posterior to posterior condylar line. Tibia torsion: positive: ankle laterally rotated to the posterior tibial plateau; negative: ankle internally rotated to the posterior tibial plateau.
Table 3
Inter- and Intraobserver Reliability of the Different Measurements

| Measurements      | Immediately after surgery | 5 months postoperatively |
|-------------------|----------------------------|-------------------------|
| **Femoral version** |                            |                         |
| Intraobserver Reliability | 0.931 (0.877–0.961) | 0.939 (0.860–0.970) |
| Interobserver Reliability | 0.912 (0.868–0.944) | 0.870 (0.807–0.917) |
| **Tibia torsion**  |                            |                         |
| Intraobserver Reliability | 0.838 (0.733–0.902) | 0.971 (0.953–0.984) |
| Interobserver Reliability | 0.838 (0.765–0.893) | 0.966 (0.947–0.979) |