Quantitative analysis of dynamic patellar tracking in patients with lateral patellar instability using a simple video system

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1. Introduction

Primary patellar dislocation has been reported that more than 40% of non-operative treatment failed in redislocation [1,2] and 55% of patients with primary dislocation would not be able to return to sports without surgery [3]. However, no standard treatment for acute and recurrent patellar dislocation has been established because the injury mechanism is complex and associated predisposing factors, which are general joint laxity, patella alta, abnormal Q angle, femoral trochlear dysplasia, laterally displaced tibial tuberosity, insufficient medial patellofemoral ligamnet (MPFL) and imbalance in the medial and lateral exrensor muscles, vary individually [4-7]. In addition, combined patellofemoral cartilage disorder [6,8,9] and advanced soft tissue injuries caused by repetitive dislocations alter the patellofemoral joint congruency, and makes the treatment strategy further complicated.

Most knees with unstable patella typically show lateral subluxation or displacement of the patella around lower knee flexion. Detection of increased lateral translation or inclination of patella in physical tests and imaging examinations is essential for diagnosis of unstable patella. Active patellar subluxation test (APS test) [10] has clinically been used for evaluation of the dynamic patellar tracking, and lateral shift and tilt of the patella during active knee extension are defined as the positive findings for unstable patella. Although it is a simple and non-invasive examination tool that does not require any special instruments or facilities, the interpretation of findings is highly examiner-dependent and the results are not shown quantitatively. In contrast, the patellar shift and tilt in reference to femoral trochlea are clinically measured on radiographs and CT with high accuracy. However, the radiation exposure is invasive to subjects and the measurements are acquired only under static conditions at limited knee flexion angles. On the other hand, various methods for quantitative evaluation of dynamic patellar tracking have been projected in previous studies; such as with custom-made devices for cadaveric specimens [11-13], dynamic CT [14], open MRI [15-19], ultrasound transducer [20] and optoelectronic motion capture [21]. However, open MRI and these original measurement systems require special facilities and equipments, and take much cost and time. The development of quantitative examination technique for dynamic patellar tracking, which is easy to use and repeatedly applicable in clinic, could help to follow-up the time-dependent changes and analyze the treatment effect on unstable patella.

For quantitative analysis of dynamic patellar tracking, we recently developed a new method using a digital video camera system, which can measure the mediolateral patellar position in reference to the knee width during active knee extension. The purposes of this study were to investigate the patellar tracking pattern of knees with patellar dislocation and the contralateral knees without patellar dislocation in patients with unstable patella, and compare them with that in healthy control subjects. We hypothesized that (1) patellar tracking pattern would be different between patients and controls, and (2) it would be different between the knee with and without patellar dislocation in patients due to the soft tissue and cartilage injuries combined with patellar dislocation.

2. Materials and methods

2-1. Patients and control subjects

Twenty-three patients (6 males and 17 females, 21.1 ± 9.4 years old) with unstable patella, who were treated in our institute from July 2011 to January 2015, were involved in this study. All patients had unilateral recurrent patellar dislocation diagnosed by the combination of history of dislocation, findings in physical examinations and on radiograph, CT and MRI. MRI was performed from 1 to 20 days after event of patellar dislocation, and the symptomatic knees in all patients showed bone bruise in the lateral femoral condyle and the medial patellar facet as an evidence of recent patellar dislocation, which was seen as a bone marrow lesion with high signal intensity in fat-suppression sequence. The contralateral knee had no history of patellar dislocation and was asymptomatic without any complaints of pain, discomfort or apprehension. The mean time period between the latest patellar dislocation to the data acquisition was 3.5 ± 2.8 months (range of 1 week - 9 months), and the mean number of dislocations was 3.0 ± 1.9 (range of 2 - 10). The patients who were unable to perform active full extension of the knee preoperatively because of pain or swelling, or had past history of any knee surgery were excluded from this study. Both the knee with patellar dislocation (dislocated group) and the contralateral knee without patellar dislocation (non-dislocated group) were investigated. No patient in this series had a hypermobile patella that was defined abnormal medial and lateral laxity in the patellar glide test [22].

For the comparative study between the patients with unstable patella and healthy controls, 23 healthy age- and sex-matched volunteers (6 males and 17 females, 20.9 ± 6.8 years old; control group) who presented no current complaints or symptoms of the knee joint, and had no previous history of patellofemoral joint problems or knee injuries were included. A unilateral knee in each control subject was randomly selected for the investigation. The study was approved by the institutional review boards of our university hospital, and written informed consent was obtained from all patients and control subjects or their guardians before their participation.

2-2. Evaluation of anatomical parameters related to unstable patella in patients

Predisposing anatomical factors for unstable patella were evaluated using plain radiographs and CT images in bilateral knees of all patients. Patella height was assessed on lateral radiographs by measuring Insall-Salvati ratio [23] and Caton-Deschamps index [24]. On the Merchant’s view, sulcus angle and congruence angle were measured for detecting trochlear dysplasia and abnormal congruence [25]. For assessing lateral displacement of tibial tuberosity and lateral patellar inclination, tibial tuberosity-trochlear groove distance (TT-TG) [2,4] and patellar tilt [26] were measured on axial CT images.

2-3. Quantitative analysis of patellar tracking in patients with unstable patella and controls

A measurement technique using digital video cameras was developed to evaluate the mediolateral position of the patella in reference to the knee width. Two large reflective surface markers (diameter 25mm) were placed on proximal to the most prominence of medial and lateral femoral epicondyles of the tested knee via an elastic band to measure the knee width (knee markers). The femoral epicondyles are generally easy to palpate and more closely identify the knee width. To record the changing position of the subject's patella during knee extension, the examiner pinched the medial and lateral edges of patella with the thumb and index fingertips on which the small reflective surface markers (diameter 14mm) were attached (patella markers) (Fig.1). Additional large markers were placed on the greater trochanter of the femur (GT), the anteroposterior center of lateral knee joint space (LJS) and the lateral malleolus (LM) (knee flexion angle markers) to measure the knee flexion angle (Fig.2). For APS test, the subject was seated on a table and ordered to actively extend the knee from hanging free position to full extension, and the examiner followed the patella by pinching with thumb and index finger. To prevent the change in position of patellar marker in reference to patella, the thumb and index fingertip were fixed on the medial and lateral edges of patella throughout each trial of APS test. The frontal and lateral views of the knee joint were simultaneously recorded with two digital video cameras (HDR-HC3, Sony, Japan) at 30Hz during APS test.

The data scanned from the digital video cameras was captured to a personal computer. Using imaging software (Dartfish software TeamPro 5.5, DARTFISH), both frontal and lateral images were synchronized, and the patellar position was measured on the frontal image and knee flexion angle on the lateral image. At first, a horizontal axis was set up on the frontal image of the knee joint (Fig.1). The lateral edge of the medial knee marker, the medial edge of lateral knee marker, and the center of the medial and lateral patella markers were projected on the horizontal axis and defined as 'Mv' point, 'Lv' point, 'mv' point and 'lv' point, respectively. And the center of the patella was defined by the midpoint between mv and lv and indicated on the horizontal axis as 'Pv' point. The mediolateral patellar position in reference to knee width (%PP) was determined by dividing the distance Mv to Pv by the distance Mv to Lv (%PP = Mv-Pv/Mv-Lv × 100). Decrease in %PP means medial translation and increase in %PP means lateral translation of the patella. On the lateral view image, the angle 'α' composed of two lines (GT-LJS and LJS-LM) was measured, and the knee flexion angle was defined as $(180 - \alpha)°$ (Fig.2). Using these synchronized data, %PP was determined consecutively from hanging free position to knee full extension. Change in %PP, which was the difference of %PP at each knee flexion angle from %PP at hanging free position, was also calculated.

2-4. Statistical analysis

All image parameters of the dislocated and non-dislocated groups were statistically compared using Mann-Whitney U test. %PP and change in %PP at every 5° from 75° to 5° of knee flexion angle in the dislocated, non-dislocated and control groups were compared respectively. All data input and calculation were performed with the SPSS ver. 22.0 (SPSS Inc., Chicago, IL, USA). *P* values of less than

0.05 were considered to be statistically significant. Both %PP and change in %PP at each knee flexion angle among the three groups were compared by analysis of variance (ANOVA) and Tukey method.

2-5. Preliminary study for the validity of the new video system

To establish the reliability of this new measurement technique using a video system, we conducted three preliminary studies prior to clinical application to determine the correlations between; (1) %PP measured with the digital video camera system and that with radiographic images acquired from a fluoroscope, (2) %PP with and without pinching patella by the examiner, and (3) %PP measured by three independent examiners. For statistical analysis, partial correlations controlling for knee flexion angle were used to assess these relationships between the data of the two groups in each study. Our power analysis showed that the smallest study populations for more than 80% of statistical power in each study were 11 in the first study and 8 in the second study.

In the first study, 12 knees of 6 healthy volunteers (1 male and 5 females, 31.5 ± 5.2 years old) were investigated to determine the effect of skin motion artifact error. The knee markers and knee flexion angle markers were placed on the landmarks described above and the subject was seated on an X-ray table. Then, APS test was performed in the same manner with markers on the fingertips of the examiner. The frontal and lateral views of knee joint during APS test was simultaneously recorded with two digital video cameras and the anteroposterior view of the knee joint was also recorded with a mobile X-ray fluoroscope. These images were captured from the digital video cameras and the fluoroscope to a personal computer and synchronized with each other. On the digital video images, %PP and knee flexion angle were measured according to the method described previously. On the image from the fluoroscope, the apex of medial and lateral femoral epicondyles and the medial and lateral edge of patella were projected on a horizontal axis. These points were defined as 'Mx' point, 'Lx' point, 'mx' point and 'lx' point, respectively. And the center of the patella was indicated as 'Px' point which was the midpoint between mx and lx. The mediolateral patellar position on radiograph (R%PP) was determined by dividing the distance between Mx to Px by the distance between Mx to Lx (R%PP = Mx-Px/Mx-Lx × 100) (Fig.3). We randomly selected 3 knee flexion angles in each of 12 knees and measured both %PP and R%PP. Statistical analysis showed that there was significant correlation between %PP and R%PP ($P < 0.001$, $r = 0.71$) (Fig.4).

In the second study, the effect of pinching patella by the examiner's fingers on patellar tracking was examined in 10 knees of 5 subjects (1 male and 4 females, 30.2 ± 4.6 years old). APS test was performed on an X-ray table with or without pinching patella by the examiner. The anteroposterior image was recorded with an X-ray fluoroscope and the lateral view for measuring the knee flexion angle was simultaneously recorded with a digital video camera. X-ray images with and without pinching were synchronized with the corresponding lateral view, and R%PP was measured at randomly selected 3 knee flexion angles in each knee. There was statistical correlation between R%PP with and without pinching the patella ($P < 0.001$, r = 0.91), indicating no significant effect of pinching patella on the mediolateral patellar position (Fig.5).

In the third study, the data were analyzed using the ICC(3, 1) to determine inter-observer reliability of measuring %PP. By 3 orthopedic surgeons, %PP at every 5° from 75° to 5° of knee flexion angle were independently measured in 16 knees of 8 control subjects (4 males and 4 females, 21.8 ± 3.3 years old). Inter-observer reliability was 0.81 (p<0.05, 95% confidence interval between 0.56 - 0.90).

3. Results

3-1. Radiological parameters for unstable patella in patients

For the dislocated and non-dislocated groups, Insall-Salvati ratio were 1.31 ± 0.15 and 1.30 ± 0.16 (p=0.930), Caton-Deschamps index were 1.14 ± 0.17 and 1.16 ± 0.17 (p=0.692), sulcus angle were 142.3 ± 8.6° and 142.4 ± 6.2° (p=0.805), congruence angle were 24.0 ± 16.2° and 14.1 ± 12.7° (p=0.033), patellar tilt were 4.0 ± 10.5° and 4.9 ± 10.7° (p=0.324) and TT-TG were 21.0 ± 4.4mm and 19.6 ± 4.0mm (p=0.425). The congruence angle in the dislocated group was significantly larger compared to the non-dislocated group, while there were no significant difference in the other parameters between the two groups (Table 1).

3-2. Patellar tracking using the video system

%PP at every 5° of knee flexion angle from 75° to 5° in three groups are shown in Figure 6 and Table 2. For the dislocated, non-dislocated and control group, %PP at 75° was 59.1 ± 5.9%, 59.0 ± 6.0% and 56.7 ± 4.4%; and %PP at 5° was 64.2 ± 5.2%, 63.8 ± 6.5% and 55.2 ± 5.2%, respectively. There were significant differences in %PP between the dislocated and control group from 30° to 5° of knee flexion angle. Also, there were significant differences in %PP between the non-dislocated and control group from 25° to 5° of knee flexion angle. No significant difference in %PP was detected between the dislocated and non-dislocated group at any flexion angles examined.

Change in %PP from 75° to 5° in the three groups are shown in Figure 7 and Table 3. Change in %PP at 5° in the dislocated, non-dislocated and control group was 5.1 ± 3.1%, 4.7 ± 3.1% and -1.1 ± 2.6%, respectively. There were significant differences in change in %PP between the dislocated and control group from 30° to 5° of knee flexion angle, and in that between the non-dislocated and control group from 25° to 5°. There was no significant difference in change in %PP between the dislocated and non-dislocated group at any flexion angles examined.

4. Discussion

In the current study, the patellar tracking pattern during active knee extension was demonstrated in patients with unstable patella and healthy subjects by measuring mediolateral patellar position using digital video cameras simply and non-invasively. In previous study, the higher coefficient of multiple correlation of patellofemoral kinematics at lower flexion angle between bone pin and skin sensors using thermoplastic patellar mold was reported [27]. In the present study, the reliability of this measurement was validated with the three preliminary studies using fluoroscopy and it was suggested that this measurement method was useful to evaluate the dynamic patellar tracking quantitatively.

The first hypothesis in this study was confirmed by the significant difference in %PP from 30° to 5° of knee flexion between patients with unstable patella and control subjects. In the previous studies, Kujala reported the first quantitative evaluation of patellar tracking with MRI in 1989 [28]. The patellar displacement of patients with recurrent patellar dislocation was examined at lower knee flexion with the quadriceps relaxed. The patella positioned more laterally at 0° than 30° of knee flexion and the displacement in patients was significantly larger than that of controls. After the first report, patellar tracking pattern during active knee extension using various measurement systems such as MRI, ultrasound under various conditions was investigated [11-21]. Our result that patellar tracking in patients with patellar dislocation was significantly larger than that of controls at lower knee flexion angle, in agreement with the previous reports.

As one of the most important findings in this study, no significant difference in %PP was detected between the dislocated and non-dislocated group at any flexion angle, not supporting the second hypothesis. MPFL is primary passive soft tissue restraint to lateral dislocation of patella and contributes 60% of the total restraining force against lateral patellar displacement. In most cases, MPFL rupture is an essential event in the first lateral patellar dislocation and it alters the patellofemoral joint instability for the worse [8,29,30]. On the other hand, it has reported that the length of MPFL in patients with patellar instability was longer than that in healthy controls, even knee with no episode of patellar dislocation [31]. And also, Regalado reported lateral patellar displacement in both of the affected and unaffected knees with unilateral patellar instability using kinematic MRI, and the patella in both knees was more lateralized with progressive extension of the knee [18]. In the present study, the radiological findings of the anatomical predisposing factors for unstable patella such as patella alta, trochlear dysplasia and lateralized tibial tuberosity were shown in the non-dislocated group as well as in the dislocated group. The similar patellar tracking pattern in the dislocated and the non-dislocated group suggested that MPFL might no longer function as a restraint to lateral instability even in the knees with no episode of patellar dislocation in patients with unstable patella.

There were several limitations in the current study. The first limitation was that this method can evaluate in vivo patellar dynamics on only a single plane and measure the patellar translation on only the mediolateral axis. Previous reports have showed patellofemoral kinematics in six degrees (anteroposterior, mediolateral, proximal-distal directions, patellar tilt, shift and spin), and it has been reported that spin and tilt was larger in patients with patellofemoral pain compared to healthy subjects [21,32,33]. The second limitation was the small sample size of patients and controls. No consider of the body frame of subjects was the third limitation. Thicker thighs in obese or well-muscled subjects might make the error on skin markers larger and the value of %PP inaccurate. The forth limitation was that patellar tracking was evaluated only during simple active knee extension under non-weight bearing. Patellar dislocation and the symptom of an unstable patella usually present during more dynamic activity with knee valgus/varus and internal/external rotation. Various patellar tracking patterns might be caused by differences in testing conditions; such as static or dynamic condition, quadriceps contraction or relaxation, weight bearing or non-weight bearing, and examination tasks. Despite these limitations, the results of current study indicated the ability of this non-invasive and simple quantitative technique to evaluate dynamic patellar tracking in clinical use.

In conclusion, the patellar tracking of patients with unstable patella and healthy control subjects were quantitatively evaluated during active knee extension using a newly developed measurement method with digital video cameras. The lateral patellar translation was observed at lower knee flexion angle in both of the knees with and without patellar dislocation in patients, showing significant difference in patellar tracking patterns compared to healthy controls.

Acknowledgment

The authors grateful thank Daisuke Chiba, MD and Eiji Sasaki, MD, Department of Orthopaedic Surgery, Hirosaki University Graduate School of Medicine, for their efforts in statistical analysis.

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Figure

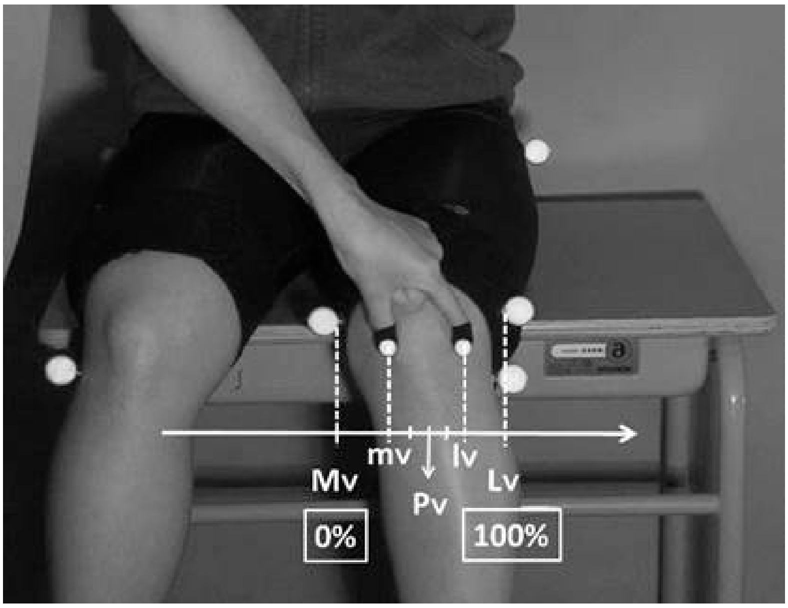

Figure 1: Measurement of mediolateral position of the patella on the video image (frontal view)

The mediolateral patellar position in reference to knee width (%PP) was defined by dividing the distance Mv-Pv by the distance Mv-Lv (%PP = Mv-Pv/Mv-Lv × 100).

Mv: medial epicondyle, Lv: lateral epicondyle, Pv: midpoint of the patella

mv: medial patella edge, lv: lateral patella edge

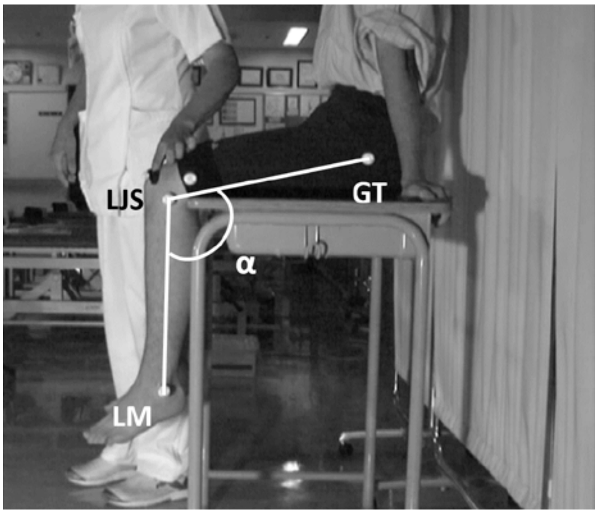

Figure 2: Measurement of knee flexion angle (lateral view)

The angle 'α' was composed of two lines (GT-LJS and LJS-LM). The knee flexion angle was defined as $(180 - \alpha)°$.

GT: greater trochanter of the femur, LJS: lateral joint space, LM: lateral malleolus

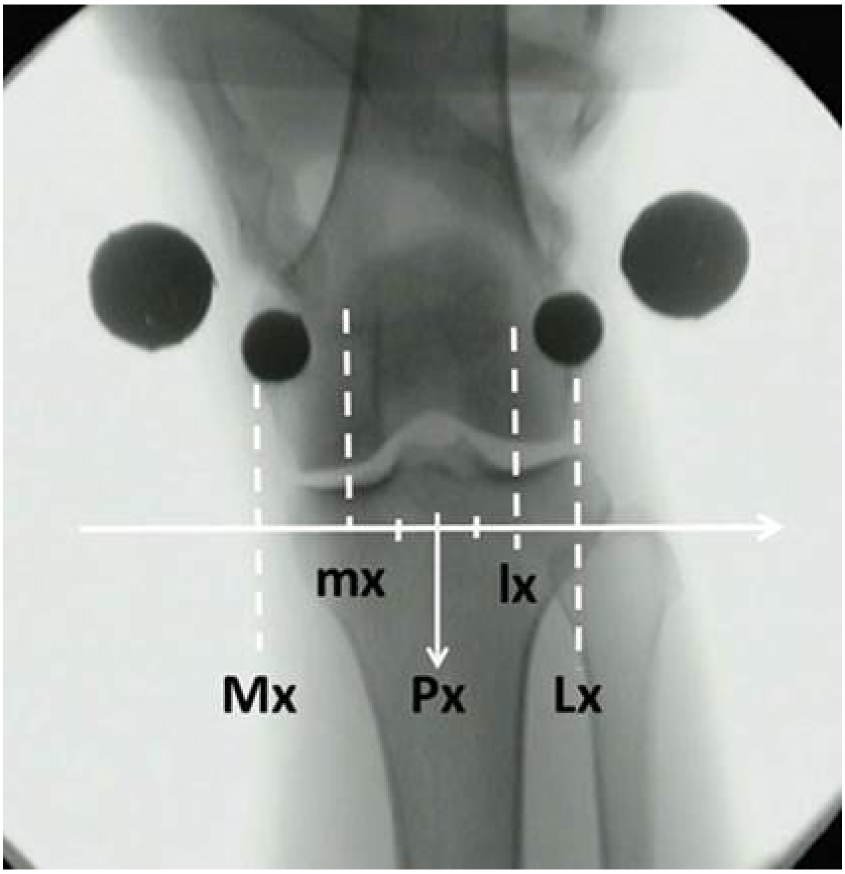

Figure 3: Measurement of mediolateral position of the patella on the fluoroscope image

The mediolateral patellar position on radiograph (R%PP) was defined by dividing the distance Mx-Px by the distance Mx-Lx (R%PP = Mx-Px/Mx-Lx × 100).

Mx: medial epicondyle, Lx: lateral epicondyle, Px: midpoint of the patella

mx: medial patella edge, lm: lateral patella edge

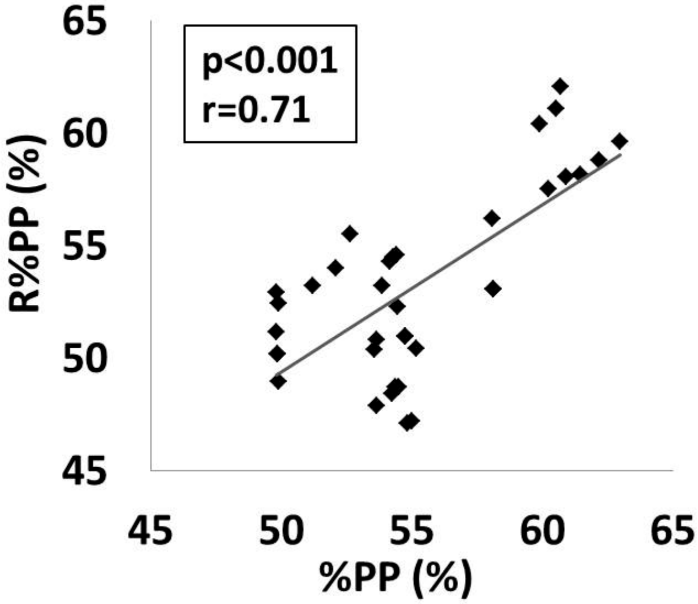

Figure 4: The correlation between %PP and R%PP

There was significant correlation between %PP and R%PP ($p < 0.001$, $r = 0.71$).

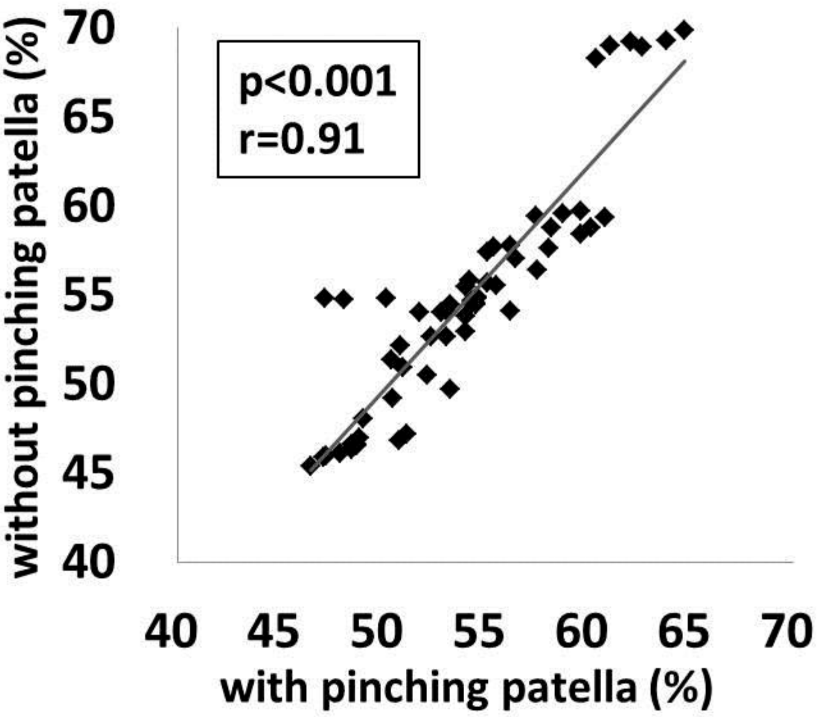

Figure 5: The correlation between R%PP with and without pinching patella

There was significant correlation between R%PP with and without pinching patella ($p < 0.001$, $r = 0.91$).

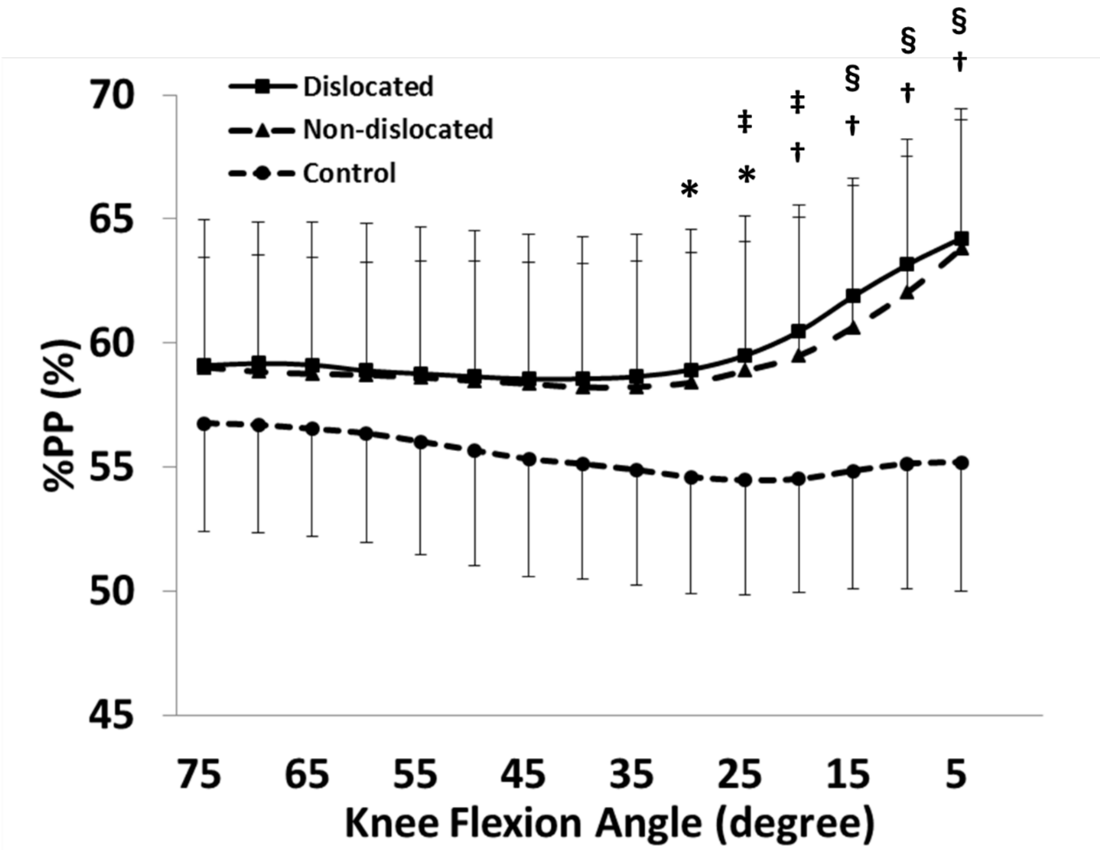

Figure 6: %PP at every 5° of knee flexion angle from 75° to 5°

There were significant differences in %PP between the dislocated and control group and between the non-dislocated and control group at lower knee flexion angle.

*$p < 0.05$, † $p < 0.01$ Dislocated vs Control

‡$p < 0.05$, §$p < 0.01$ Non-dislocated vs Control

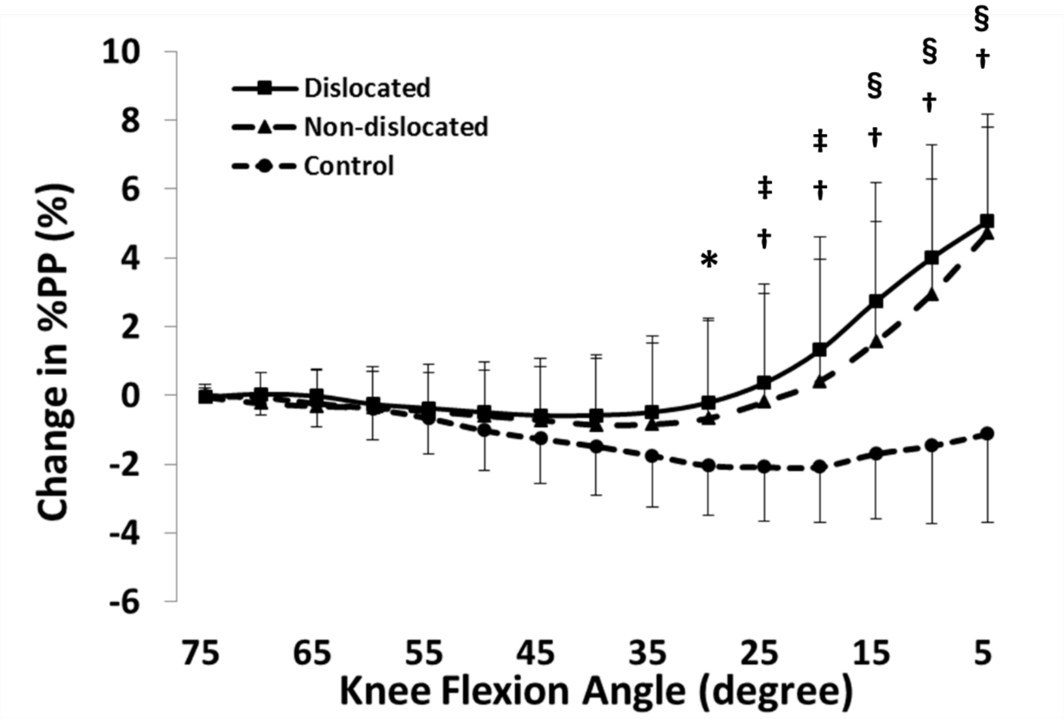

Figure 7: Change in %PP at every 5° of knee flexion angle from 75° to 5°

There were significant differences in change in %PP between the dislocated and control groups and between the non-dislocated and control groups at lower knee flexion angle.

*$p < 0.05$, † $p < 0.01$ Dislocated vs Control

‡$p < 0.05$, §$p < 0.01$ Non-dislocated vs Control

Table 1: Radiographic measurements for each group

	Dislocated	Non-dislocated	*P*
Insall-Salvati ratio	1.31 ± 0.15	1.30 ± 0.16	.930
Caton-Deschamp index	1.14 ± 0.17	1.16 ± 0.17	.692
Sulcus angle (deg)	142.3 ± 8.6	142.4 ± 6.2	.805
Congruence angle (deg)	24.0 ± 16.2	14.1 ± 12.7	.033
Patellar tilt (deg)	4.0 ± 10.5	4.9 ± 10.7	.324
TT-TG distance (mm)	21.0 ± 4.4	19.6 ± 4.0	.425

Table 2:

	Dislocated			Non-dislocated			Control	
	ave ± SD		(95%CI)	ave ± SD		(95%CI)	ave ± SD	(95%CI)
75	59.1 ± 5.9		(56.5 − 61.7)	59.0 ± 6.0		(56.4 − 61.6)	56.7 ± 4.4	(54.9 − 58.6)
70	59.2 ± 6.0		(56.6 − 61.8)	58.8 ± 6.0		(56.3 − 61.4)	56.7 ± 4.4	(54.8 − 58.6)
65	59.1 ± 6.1		(56.5 − 61.8)	58.8 ± 6.1		(56.1 − 61.4)	56.5 ± 4.3	(54.7 − 58.4)
60	58.9 ± 6.1		(56.2 − 61.5)	58.7 ± 6.2		(56.0 − 61.4)	56.3 ± 4.4	(54.5 − 58.2)
55	58.8 ± 6.1		(56.1 − 61.4)	58.6 ± 6.3		(55.9 − 61.3)	56.0 ± 4.5	(54.0 − 58.0)
50	58.6 ± 6.0		(56.0 − 61.3)	58.5 ± 6.3		(55.7 − 61.2)	55.7 ± 4.7	(53.6 − 57.7)
45	58.5 ± 6.0		(55.9 − 61.1)	58.4 ± 6.4		(55.6 − 61.1)	55.3 ± 4.7	(53.3 − 57.4)
40	58.6 ± 6.1		(55.9 − 61.2)	58.2 ± 6.6		(55.4 − 61.0)	55.1 ± 4.7	(53.1 − 57.1)
35	58.6 ± 6.1		(56.0 − 61.3)	58.2 ± 6.9		(55.3 − 61.2)	54.9 ± 4.7	(52.9 − 56.9)
30	58.9 ± 6.2	*	(56.2 − 61.6)	58.4 ± 7.1		(55.3 − 61.5)	54.6 ± 4.7	(52.6 − 56.6)
25	59.5 ± 6.2	*	(56.8 − 62.2)	58.9 ± 7.1	*	(55.8 − 62.0)	54.5 ± 4.6	(52.5 − 56.5)
20	60.5 ± 6.1	†	(57.8 − 63.1)	59.5 ± 7.3	*	(56.3 − 62.7)	54.5 ± 4.6	(52.5 − 56.5)
15	61.9 ± 5.7	†	(59.4 − 64.6)	60.6 ± 7.2	†	(57.5 − 63.8)	54.8 ± 4.8	(52.8 − 56.9)
10	63.2 ± 5.5	†	(60.8 − 65.5)	62.0 ± 6.9	†	(59.1 − 65.0)	55.1 ± 5.1	(52.9 − 57.3)
5	64.2 ± 5.2	†	(62.0 − 66.5)	63.8 ± 6.5	†	(61.0 − 66.6)	55.2 ± 5.2	(52.9 − 57.5)

*$p < 0.05$, † $p < 0.01$ compared to Control

Table 3: Change in %PP at every 5° of knee flexion angle from 75° to 5

	Dislocated			Non-dislocated			Control	
	ave ± SD		(95%CI)	ave ± SD		(95%CI)	ave ± SD	(95%CI)
75	0.0 ± 0.3		(-0.2 − 0.1)	-0.1 ± 0.4		(-0.2 − 0.1)	0.0 ± 0.2	(-0.1 − 0.1)
70	0.0 ± 0.6		(-0.2 − 0.3)	-0.2 ± 0.9		(-0.6 − 0.2)	-0.1 ± 0.5	(-0.3 − 0.2)
65	0.0 ± 0.8		(-0.4 − 0.3)	-0.3 ± 1.0		(-0.8 − 0.1)	-0.2 ± 0.7	(-0.5 − 0.1)
60	-0.3 ± 1.0		(-0.7 − 0.2)	-0.4 ± 1.2		(-0.9 − 0.2)	-0.4 ± 0.9	(-0.8 − 0.0)
55	-0.4 ± 1.0		(-0.8 − 0.1)	-0.5 ± 1.4		(-1.0 − 0.1)	-0.7 ± 1.1	(-1.1 − -0.2)
50	-0.5 ± 1.2		(-1.0 − 0.0)	-0.6 ± 1.6		(-1.3 − 0.1)	-1.0 ± 1.2	(-1.5 − -0.5)
45	-0.6 ± 1.4		(-1.2 − 0.0)	-0.7 ± 1.8		(-1.5 − 0.1)	-1.3 ± 1.3	(-1.8 − -0.7)
40	-0.6 ± 1.7		(-1.3 − 0.1)	-0.9 ± 2.0		(-1.7 − 0.0)	-1.5 ± 1.4	(-2.1 − -0.9)
35	-0.5 ± 2.0		(-1.4 − 0.4)	-0.8 ± 2.6		(-2.0 − 0.3)	-1.7 ± 1.5	(-2.4 − -1.1)
30	-0.2 ± 2.4	*	(-1.3 − 0.8)	-0.7 ± 2.8		(-1.9 − 0.6)	-2.0 ± 1.4	(-2.7 − -1.4)
25	0.4 ± 2.9	†	(-0.9 − 1.6)	-0.2 ± 3.1	*	(-1.5 − 1.2)	-2.1 ± 1.6	(-2.8 − -1.4)
20	1.3 ± 3.3	†	(-0.1 − 2.7)	0.4 ± 3.5	*	(-1.1 − 1.9)	-2.1 ± 1.6	(-2.8 − -1.4)
15	2.7 ± 3.4	†	(1.2 − 4.2)	1.6 ± 3.5	†	(0.1 − 3.1)	-1.7 ± 1.9	(-2.5 − -0.9)
10	4.0 ± 3.3	†	(2.6 − 5.4)	3.0 ± 3.3	†	(1.5 − 4.4)	-1.5 ± 2.3	(-2.4 − -0.5)
5	5.1 ± 3.1	†	(3.7 − 6.4)	4.7 ± 3.1	†	(3.4 − 6.1)	-1.1 ± 2.6	(-2.3 − 0.0)

*p < 0.05, † p < 0.01 compared to Control