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Research Article

Prevalence of Cartilage Erosion in Canine Patellar Luxation and Gene Expression in Affected Joints

Burin BOONSRI [1] Waranee PRADIT [2] Kumpanart SOONTORNVIPART [3] Terdsak YANO [4]
Siriwadee CHOMDEJ [2] Siriwan ONGCHAI [5] Korakot NGANVONGPANIT [1]

[1] Animal Bone and Joint Research Laboratory, Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, THAILAND
[2] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, THAILAND
[3] Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, THAILAND
[4] Department of Food Animals, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, THAILAND
[5] Thailand Excellence Center for Tissue Engineering, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, THAILAND

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Abstract

The objectives of this study were to assess the prevalence of cartilage erosion in small dogs with patellar luxation (PL), and related osteoarthritis (OA)-related gene expression. In Study 1, 71 dogs were examined to determine risk factors associated with PL, including breed, age, weight, sex, and affected joint. In Study 2, a total of 39 dogs were divided into four groups: normal articular cartilage in the stifle joint (G1; n=5); PL without cartilage erosion (G2; n=11); PL with cartilage erosion (G3; n=14); and OA in the stifle (G4; n=9). Articular cartilage and synovial membranes were collected during surgical operations to correct PL. Real-time PCR was used to quantify the expression levels of 11 OA-related genes, including *AGG, COL2A1, HAS-1, HAS-2, TIMP-1, MMP-3, IL-1β, TNF-α, IFN-γ, COX-1,* and *COX-2*, with *GAPDH* used as a reference gene. From Study 1, it was found that the risk factors related with cartilage erosion lesion were age, sex, and PL grade (all variables showed $P<0.05$). From Study 2, it was demonstrated that PL with or without cartilage erosion expressed pro-inflammatory cytokines and enzymes; some biomolecules were up regulated *(IL-1β, MMP-3, AGG, TIMP-1)* but some were down regulated *(COL2A1, HAS-2, COX-1, COX-2)*. This expression was the difference between the articular cartilage and the synovial membrane; however, the expression of genes from PL with cartilage erosion was observed to be similar to that of OA. From our results, it can be concluded that PL can develop into secondary OA due to an increase of *IL-1β* in cartilage and synovial membrane.

Keywords: *Cartilage erosion, Dog, Gene expression, Patellar luxation*

Köpeklerde Patellar Luksasyonda Kıkırdak Erozyonunun Prevalansı ve Etkilenmiş Eklemlerdeki Gen Ekspresyonu

Özet

Bu çalışmanın amacı patellar luksasyonlu (PL) küçük cüsseli köpeklerde kartilaj erozyonunun prevalansını ve osteoartritis (OA)-alakalı gen ekspresyonunu belirlemektedir. Birinci araştırmada; cins, yaş, cinsiyet ve infekte eklemi içeren PL ile ilgili risk faktörlerini belirlemek amacıyla 71 köpek incelendi. İkinci araştırmada toplam 39 köpek dört gruba ayrıldı; diz ekleminde normal artikular kartilaj (G1; n=5), kartilaj erozyon olmayan PL (G2; n=11), kartilaj erozyonlu PL (G3; n=14) ve dizde OA (G4; n=9). Cerrahi operasyon sırasında PL'u düzeltmek amacıyla artiküler kartilaj ve sinoviyal zarlar alındı. Toplam 11 adet OA ile ilgili genin *(AGG, COL2A1, HAS-1, HAS-2, TIMP*-1, *MMP-3, IL-1β, TNF-α, IFN-γ, COX-1* ve *COX-2*; referans gen olarak GAPDH kullanıldı) ekspresyon düzeylerini belirlemek amacıyla PCR tekniği uygulandı. Birinci araştırmanın sonucunda yaş, cinsiyet ve PL derecesi kartilaj erozyonu ile ilgili risk faktörleri olarak belirlendi ($P<0.05$). İkinci araştırmada kartilaj erozyonlu veya erozyon bulunmayan PL'lu köpeklerde pro-inflamatuar sitokinleri eksprese ettikleri, bazı biyomoleküllerin ekspresyonunu artırdıkları *(IL-1β, MMP-3, AGG, TIMP-1)* bazılarını ise azalttıkları (IL-1β, MMP-3, AGG, TIMP-1) gözlemlendi. Bu ekspresyon artiküler kartilaj ve sinoviyal zar için farklıydı. Ancak kartilaj erozyonlu PL'da genlerin ekspresyonu OA ile benzerlik göstermekteydi. Çalışma bulguları doğrultusunda kıkırdak ve sinoviyal zarlarda artan *IL-1β*'ya bağlı olarak PL'un sekonder OA'e yol açabileceği sonucuna varıldı.

Anahtar sözcükler: *Kartilaj erozyonu, Köpek, Gen ekspresyonu, Patella çıkığı*

İletişim (Correspondence)
+66 5394 8046
korakot.n@cmu.ac.th

INTRODUCTION

Patellar luxation (PL) is one of the most common joint diseases in small breed dogs [1-3], the prevalence of which has been studied worldwide. In Chiang Mai, Thailand, for example, 128 out of 317 dogs (40.3%) were reported to be affected with PL, predominantly in poodles (34.4%), Pomeranians (28.9%), and Chihuahuas (12.5%). Even in the United States, a study found that 43% of Pomeranians and 23.6% of Dutch flat-coated retrievers had PL [4]. Originally, clinicians focused on finding an effective surgical technique for treating this condition [5-8]. So far, targeted gene studies have detected loci on chromosomes 7 and 31 that are involved in PL [2,9,10]. Patellar luxations can be medial or lateral and are graded based on severity [11]. A higher grade of PL is associated with certain joint diseases, such as cranial cruciate ligament rupture [1,12].

Osteoarthritis (OA) is one of the most common joint diseases in animals, and in humans as well. Many joint diseases in dogs have been proven to be the cause of OA, such as cranial cruciate ligament rupture [13-15], meniscus injury [14,15], elbow dysplasia [16], and hip dysplasia [17]. Although a relationship between PL and OA has not been well established in dogs or in humans, three reports have indicated that PL is a possible cause. The other study also reported significant potential in treating PL without surgery [18]. Patellar luxation causes joint instability from the lateral or medial movement of the patella on the femoral groove. The movement of the patella in and out of the femoral groove in PL can cause cartilage erosion [19-21], which may then develop into OA [18,22,23].

As yet, PL has not been reported to be associated with expression of OA-related genes in dogs or in humans. This work aims to study the expression of some OA-related genes from articular cartilage and synovial membrane in canine PL. The objectives of this study were to determine the prevalence of cartilage erosion in PL and to compare the expression of genes in PL, with or without cartilage erosion, to OA gene expression. The hypothesis is, if PL is related to development of OA, then the expression of some OA-related genes will differ from normal and be similar to OA joint. Additionally, we study the incidence of cartilage erosion in PL.

MATERIAL and METHODS

This research consisted of two independent studies. The first is retrospective data showing the prevalence of cartilage erosion in canine PL. The second study investigated the expression level of some OA-related genes from the articular cartilage and the synovial membrane. The Ethics Committee of the Faculty of Veterinary Medicine, Chiang Mai University, Thailand, approved this study in 2014.

Study 1: Incidence of Cartilage Erosion in Canine

Animals: The data *-including breed, age, weight, sex, and affected stifle joint- were recorded from 71 PL dogs (Table 1)* that had visited the Animal Hospital for stifle surgery from 2010 to 2014.

Patellar Luxation Grading: The degrees of PL were classified into four grades, as determined by manipulation [1,24,25]. *Grade I:* The patella can be luxated from the femoral groove when the stifle was fully extended and the patella can return into the femoral groove immediately. *Grade II:* The patella moves out of the femoral groove for sometime, but it can return to the normal position spontaneously. *Grade III:* The patella is normally luxated from the femoral groove but can be returned to a normal position by manipulation. *Grade IV:* The patella is permanently luxated from the femoral groove and cannot returned to this normal position.

Cartilage Erosion Lesion: During exploratory stifle arthrotomy, the lesions on six anatomical sub-regions of articular cartilage on the patella and femoral trochlea, including the central patella, medial patella, lateral patella, medial trochlea, lateral trochlea, femoral groove, and osteophyte formation were examined and evaluated as positive or negative lesions.

Statistical Analysis: Age and weight were reported as mean±SD, sex was reported in terms of number of male and female, and affected joints were reported in terms of percentage. The prevalence of cartilage erosion lesions in canine PL cases was reported with 95% confidence interval (95% CI). The relationship between degrees of PL and positive/negative cartilage erosion was analyzed using Fisher's exact test. The R statistical program was used to analyze the risk factors of cartilage erosion lesion finding. Univariable analysis was performed using Fisher's exact test. A thres-hold value of $P<0.05$ was used to screen variables for the multivariable model. A multivariable logistic regression model was used to assess the risk factors of this outcome. The Akaike information criterion was used to select the best-constructed model. The receiver operating characteristic curve was tested to evaluate model accuracy.

Table 1. *Information on patients included in study 1*
Tablo 1. *Birinci araştırmadaki hasta bilgileri*

Breed	Number	Age (months) min-max (mean)	Weight (kg) mean±SD	Sex	
				Male	Female
Pomeranian	33	6-120 (37)	3.7±1.6	15	18
Chihuahua	22	8-96 (25)	2.7±1.5	7	15
Shih Tzu	6	36-84 (49)	5.8±1.2	3	3
Yorkshire Terrier	5	18-96 (40)	2.4±0.8	4	1
Poodle	5	24-120 (64)	5.1±2.3	2	3

Study 2: Articular Cartilage and Synovial Membrane Gene Expression in Canine Patellar Luxation

Animals: A total of 39 dogs were divided into four groups *(Table 2)*: G1=dogs without gross evidence of pathology of articular cartilage from the stifle joint (n=5); G2=PL without cartilage erosion (n=11); G3=PL with cartilage erosion (n=14); and G4=dogs with stifle OA (n=9), when OA lesions were present in the joint, based on the following criteria: cartilage fibrillation, erosion, and osteophytes [26].

Inclusion/Exclusion Criteria for Samples: The dogs belonging to G2 and G3 were small-breed dogs less than 5 years old with clinical signs of medial PL. Animals that were pregnant, were with neurological disease, or had undergone musculoskeletal surgery were excluded. Dogs with lameness due to cranial cruciate ligament rupture or meniscal injury, and those with nerve injury, lumbosacral instability, infection, immune diseases, and fractures were also excluded. Dogs belonging to G1 had visited the hospital for hind limb amputation (from a traffic accident). Gross pathology reports of previously collected cartilage and synovial membrane were evaluated, and it was confirmed that the reports did not show cartilage and synovial membrane lesions. Moreover, these dogs had no documented history of stifle disorder. Dogs in G4 were diagnosed with cranial cruciate ligament rupture or meniscus injury at least 1 month prior to surgery; moreover, this group was free from PL. Samples of articular cartilage and synovial membrane were collected during the operation.

Patellar Luxation and Articular Cartilage Erosion: The degrees of medial PL were classified into four grades by manipulation, as mentioned previously. Dogs with medial PL had been recommended to undergo surgical correction of the condition by a veterinarian. Articular cartilage erosion was evaluated during the operation. The criterion of cartilage erosion was applied from macroscopic scoring of femoral condyle and patella as mention in Cook et al.[27]. Cartilage erosion was classified as either positive or negative.

Collected Cartilage and Synovial Membrane: During stifle the operation, the cartilage was collected at the lateral site of the femoral trochlea using a scalpel blade, while the synovial membrane was collected at the incision site immediately following arthrotomy *(Fig. 1)*. For dogs in G1 and G4, samples were collected at the same site as for G2 and G3, to avoid the location of articular cartilage being a factor in the analysis. The size of each sample of the cartilage and the synovial membrane was approximately 0.5 cm in length. The samples were ground and homogenized with TRIzol® reagent for RNA isolation [28,29]. Due to ethical considerations, sample collection was performed so that the procedure itself would not cause OA or other joint disease. Cartilage samples could not be collected at cartilage lesions because of the possibility that this could lead to progressive OA [30]. A previous study found that collection of cartilage at the lateral site of the femoral trochlea would not cause OA [31].

Gene Expression Analysis: The genes involved in OA were investigated for their expression levels in the cartilage, and the synovial membrane tissues collected from dogs with OA and PL, using the quantitative real-time PCR method. The tissues were evaluated for the expression level of 11 genes, as follows: 5 anabolic-related genes: aggrecan *(AGG)*, collagen type II alpha 1 *(COL2A1)*, hyaluronan synthase 1 *(HAS-1)*, hyaluronan synthase 2 *(HAS-2)*, and the tissue inhibitor of metalloproteinase 1 *(TIMP-1)*; 1 catabolism-relatedgene:matrix metalloproteinase 3 *(MMP-3)*; 3 pro-inflammatory cytokine genes: interleukin 1 beta *(IL-1β)*, tumor necrosis factor alpha *(TNF-α)*, and interferon gamma *(IFN-γ)*; and 2 inflammatory enzyme genes: cyclooxygenase 1 *(COX-1)* and cyclooxygenase 2 *(COX-2)*. Glycer-

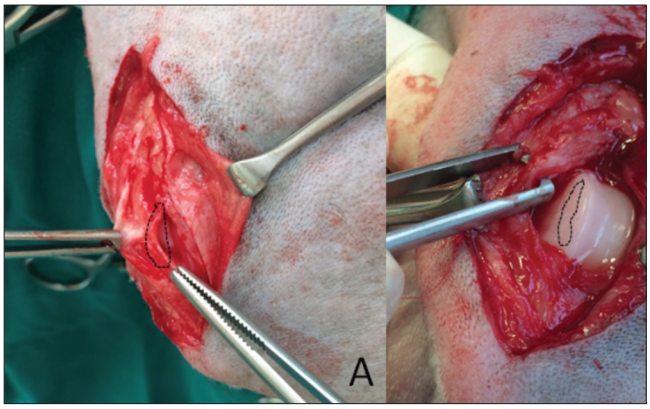

Fig 1. The excision sites of (A) synovial membrane and (B) articular cartilage

Şekil 1. Sinoviyal zar (A) ve artikular kartilajdaki (B) kesit alanları

Table 2. *Information on patients included in study 2*
Tablo 2. *İkinci araştırmadaki hasta bilgileri*

Groups	Articular Cartilage	Number Total Number (male:female)	Age (months) min-max (mean)	Weight (kg) mean±SD
G1	Normal	5 (2:3)	12-58 (34)	1.81±4.67
G2	Patellar luxation without cartilage erosion	11 (7:4)	6-60 (20)	3.76±2.37
G3	Patellar luxation with cartilage erosion	14 (3:11)	6-60 (20)	3.76±2.37
G4	Osteoarthritis	9 (4:5)	24-120 (67)	7.33±5.32

aldehyde-3-phosphate dehydrogenase *(GAPDH)* was used as the endogenous control gene (reference gene) [28].

RNA Isolation, cDNA Synthesis and Quantitative Real-Time PCR: The total RNA of the cartilage and the synovial membrane were isolated by using aninnuPREP DNA/RNA Mini Kit (Analytik Jena AG, Germany), as described in the manufacturer's protocol. -Reverse transcription of the total RNA from the cartilage and the synovial membrane was carried out to synthesize first-strand cDNA. The Expression of the genes related to OA was measured by quantitative real-time PCR by using an Eco™ Real-Time PCR System (Illumina, USA). The PCR reaction was incubated according to the following protocol: 95°C for 10 min, 45 cycles of denaturation at 95°C for 20 s, annealing at different annealing temperatures *(Table 3)* for 15 s, and extension at 72°C for 15 s. The relative expressions were calculated using threshold cycles (C_T) with normalization to the reference gene (*GAPDH*) [28].

Statistical Analysis: The amplification efficiency of genes reported to the GAPDH expression as the internal control. The mRNA level, expressed as Ct, ΔCt (Ct gene - Ct GAPDH), was used to calculate the relative quantification (Rq), using $2^{-\Delta\Delta Ct}$ methods. The expression of the control group (G1) served as a reference (Rq=1). The data were presented as box plots and statistically analyzed using the SPSS 17 software. The expression level difference groups were determined using ANOVA and multiple comparison tests. $P<0.05$was considered to be statistically significant.

RESULTS

Prevalence of Cartilage Erosion in Canine Patellar Luxation: Out of a total of 71 dogs surveyed for cartilage erosion, 39% (28/71) demonstrated cartilage lesions predominantly on the femoral trochlea and patella. The majority of these animals (24/28) had one lesion, whereas three animals had two, and one had three lesions on the articular surfaces and bone *(Table 4)*. Out of 33 lesions observed, the majority were observed on the medial patella (30% of total lesions, 10/33), while 27% (9/33), 21% (7/33), 18% (6/33), and 3% (1/33) were observed on the lateral patella, the center patella, the medial trochlea, and lateral trochlea osteophytes, respectively *(Fig. 2)*.

Cartilage erosion was not found on the femoral groove or the lateral trochlea. On five stifles (18%) were found two lesions; four stifles showed lesion on the medial and the lateral patella, one stifle revealed lesion on the center of the patella and osteophyte on the lateral trochlea. Three lesions were found on one stifle (3%) on the medial and the lateral patella as well as the medial trochlea. Risk factors that were related to cartilage erosion were age, sex, and PL grade ($P<0.05$). The prevalence of cartilage erosion was higher (OR = 7.05, $P<0.05$) in female dogs compared with male dogs and increased with age (OR = 1.04, $P<0.05$).

Gene Expression: The quantity and quality of the cDNA samples synthesized from the RNA was evaluated using a spectrophotometer (Biodrop Ltd., Cambridge, UK). It was

Table 3. *Sequences of primers used in quantitative real-time PCR*
Tablo 3. *Kantitatif gerçek zamanlı PCR'da kullanılan primer sekansları*

Gene	Primer Sequence (5'→3')	Length (bp)	Accession Number	Annealing Temperature (°C)
GAPDH	Fw: AGTATGATTCTACCCACGGC Rw: CGAAGTGGTCATGGATGACT	362	DQ403060	55
MMP-3	Fw: CTCACCCAGCAATACCTAGA Rw: CAGAGCTTTCTCAATGGCAG	318	AY183143_1	57
TIMP-1	Fw: ATCCTGCTGTTGCTGTGG Rw: GTCGGTCTGGTTGACTTCTGC	138	NM_001003182	57
AGG	Fw: GCCACCATCAGAAACCTAC Rw: AGACACCTCGGAAGCAGA	350	NM_001113455	57
COL2A1	Fw: CAGCGAGCGTTCCCAAGA Rw: CAGGCGGAGGAAGGTCAT	158	NM_001006951	60
HAS-1	Fw: CAGACACGCTGGTCCAAATC Rw: GCATAGAAGAGCCGCAACAC	149	XM_849398	55
HAS-2	Fw: GGTCATAGATGGGAACTCG Rw: GACTCATCCGTCTCACCAG	135	XM_539153	51
TNF-α	Fw: AGTGCCGTCAGATGGGTTG Rw: CCAGGTAGATGGGCTCGTA	215	NM_001003244	58
IFN-γ	Fw: AGGTCCAGCGCAAGGCGATA Rw: TCGATGCTCTGCGGCCTCGAA	117	NM_001003174	60
IL-1β	Fw: CACAGGTTCTCTGGTAGATGAGG Rw: TGGCTTATGTCCTGTAACTTGC	264	Z70047.1	50
COX-1	Fw: GGATGGAGAGATGTACCCGC Rw: CCCAATGAGGATGAGTCGGG	244	NM_001003023.2	60
COX-2	Fw: GGGAACTCCGCCGCGA Rw: CCGTAGAATCCTGTTCGGGT	167	NM_001003354.1	55

found that cDNA concentrations ranged from 0.6 to 2.3 µg/µl. The purities of the OD 260/280 and 260/230 ratios were 1.6-1.7 and 1.7-2.8, respectively.

Cartilage Gene Expression: The expressions of all 11 genes in cartilage are shown in *Fig. 3*. In comparing the OA group (G4) to controls (G1), seven transcripts were expressed to a lower degree in G4 ($P<0.05$), which included *HAS-1, HAS-2, COL2A1, AGG, IFN-γ, COX-1,* and *COX-2*, while the other four transcripts had higher expression in G4. Only *MMP-3* and *IL-1β* had higher ($P<0.05$) expression in G1 than in G4. The expressions of *COL2A1* and *IFN-γ* were highly expressed ($P<0.05$) in G2 compared with G3. In G2, the expressions of *HAS-1, HAS-2 AGG, TIMP-1, MMP-3,* and *TNF-α* did not differ ($P>0.05$); however, expressions of *IL-1β* and *IFN-γ* was higher ($P<0.05$) compared with G1. Between G2 and G4, expression of *HAS-1, HAS2, COL2A1, AGG, IFN-γ, COX-1,* and *COX-2* were higher ($P<0.05$) in G2, while in G3, the expression of *TIMP-1, MMP-3, IL-1*β, COX-2, and *TNF-α* were not different ($P>0.05$), nor were did they differ compared to G1. The expressions of *AGG, IFN-γ,* and *COX-1* were higher ($P<0.05$) in G3 compared with G4. Last, expression of *MMP-3* was lower ($P<0.05$) in G3 compared with G4.

Synovial Membrane Gene Expression: The expressions of genes from the synovial membrane are shown in *Fig. 4*. Comparing G1 and G4 groups, two genes, namely *HAS-1* and *HAS-2,* were expressed to a lower extent ($P<0.05$) in G4, whereas *IL-1β* and *COX-1* were similar ($P>0.05$). Three genes, which included *TNF-α*, *IFN-γ,* and *COX-2,* had higher expression ($P<0.05$) in G4. Between G2 and G3, *TIMP-1* expression was higher ($P<0.05$) in G3, whereas *COL2A1, MMP-3, COX-2, IFN-γ* and *TNF-α* was not ($P>0.05$). *IL-1β* expression was lower ($P<0.05$) in G3, but *HAS-1, HAS-2, AGG,* and *COX-1* expression was similar ($P>0.05$). *TIMP-1* in G2 was expressed more ($P<0.05$) than that in G1, while *HAS-1, COL2A1, AGG, MMP-3, IL-1β, TNF-α,* and *IFN-γ* had similar expression levels ($P>0.05$). Notably, *HAS-2* showed lower ($P<0.05$) expression compared with G1. The expression of *HAS-2, AGG,* and *IL-1β* in G2 was higher ($P<0.05$), while *TIMP-1, TNF-α, IFN-γ* and *COX-2* was lower ($P<0.05$) in comparison with G4. *MMP-3* in G3 showed higher ($P<0.05$) expression in comparison with G1, while *HAS-1, COL2A1, AGG, TIMP-1, IL-1β, COX-2,* and *TNF-α* did not show a difference ($P>0.05$) inexpression. Lower epression of *HAS-2* ($P<0.05$), but not *COX-1* or *COX-2* ($P>0.05$), was observed in G3 as compared to G1. *AGG* and *TIMP-1* in G3 showed higher ($P<0.05$) expression, while *COX-2* showed no difference ($P<0.05$) in expression in comparison with G4.

DISCUSSION

The relationship between cartilage erosion and PL in humans has been widely reported [19,20,32], and a few studies have been performed in dogs [21,23]. In the reports on humans, a high prevalence of cartilage lesions, of 40-97%, has been reported [19,20,32], while in dogs, prevalence reported has been 39.5% [21]; in addition, our study reports the percentage of prevalence in dogs to be 39%.

Our study also found that the grade of PL had an effect on cartilage erosion ($P<0.05$). However, the odds ratio (OR) cannot be reported because of the low number of dogs in each group of patellar grade, making the OR number of this factor extremely high. A larger number of dogs is needed in each group for finding the OR number of the patellar grade and the cartilage erosion. A total of 79% of the cartilage erosion in PL was found in a single location, 18% was observed in two locations, and 3% was found in three locations. These findings are in accordance with a previous report [21] which demonstrated that very high percentages of cartilage erosion were found in 21-60% of the examination areas. The reason that we did not conduct the evaluation in terms of areas of cartilage erosions because during the operation it is not possible to do a measurement of the actual area of all the articular cartilage, precise measurement of the area was not possible at the time of surgery. In our study, low grades of PL (1-2) were

Table 4. *Percentage of positive and negative cartilage lesions as well as location of cartilage lesions in 71 cases of patellar luxation*
Tablo 4. *Patella çıkıklı 71 olgudaki lezyonlar*

Patella Grade	Cartilage Lesions (total stifle joints = 71)			Cartilage Lesions in Different Sub-regions (total lesions = 33)						
	Total	Pos.	Neg.	A	B	C	D	E	F	G
1	14% (10/71)	0% (0/28)	23% (10/43)	0% (0/33)	0% (0/33)	0% (0/33)	0% (0/33)	0% (0/33)	0% (0/33)	0% (0/33)
2	13% (9/71)	0% (0/28)	21% (9/43)	0% (0/33)	0% (0/33)	0% (0/33)	0% (0/33)	0% (0/33)	0% (0/33)	0% (0/33)
3	37% (26/71)	29% (8/28)	42% (18/43)	12% (4/33)	0% (0/33)	6% (2/33)	6% (2/33)	0% (0/33)	0% (0/33)	0% (0/33)
4	37% (26/71)	71% (20/28)	14% (6/43)	18% (6/33)	27% (9/33)	15% (5/33)	12% (4/33)	0% (0/33)	0% (0/33)	3% (1/33)
Total	71	39% (28/71)	61% (43/71)	30% (10/33)	27% (9/33)	21% (7/33)	18% (6/33)	0% (0/33)	0% (0/33)	3% (1/33)

Pos. = positive, Neg. = negative, A = medial patella, B = lateral patella, C = center patella, D = medial trochlea, E = lateral trochlea, F = femoral groove, G = osteophyte

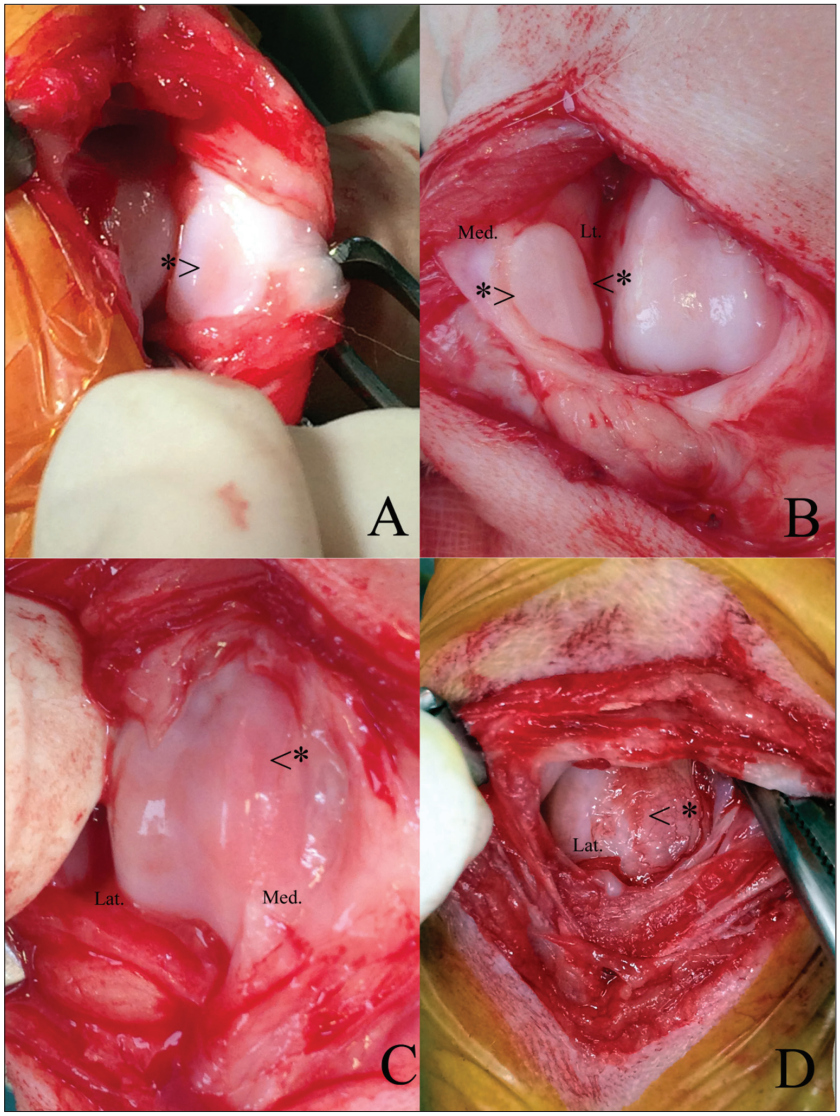

Fig 2. A representative photo of cartilage erosion on the (A) center of patella, (B) medial and lateral patella, (C) medial trochlea, and (D) osteophyte (Lat. = lateral; Med. = medial)

Şekil 2. Patellanın merkezinde (A), medial ve lateralinde (B), medial trocleada (C) ve osteohytede (D) kartilaj erozyonlarının göstüntüsü (Lat. = lateral; Med. = medial)

not found to be affected with cartilage erosion, while a report from Daems et al.[21] demonstrated 55% and 42% from grade 1 and grade 2 as being affected with cartilage erosion; the contrasting reports may be due the difference in the sizes of dogs in the two studies. The study conducted by Daems et al.[21] included all breed sizes (small to giant breeds) with weights in the range of 1-42 kg (the median weight being 8.8 kg), while our study included only small breeds with weights in the range of 1.2-9.0 kg (the median weight being 3.5 kg). The conclusion arrived at by Daems et al.[21] suggests a weak significant correlation between cartilage erosion and body weight, but taken together with the findings in this study, it may be possible that weight does have an effect on cartilage erosion in PL. Moreover, our first study found the prevalence of cartilage erosion to be significantly higher in female dogs compared with males. Additionally, with increasing age, the prevalence of cartilage erosion was also found to increase significantly. It has been well documented in many publications that aging has an effect on cartilage morphology and biology, which can cause OA [33,34].

In the process of harvesting articular cartilage, we collected at the lateral aspect of the femoral trochlea in all four groups. From a previous study on chondrocyte transplant, including research done by our team [31], this location is the best for harvesting articular cartilage without causing OA. Moreover, this is in accordance with ethical standards, whereby any clinical research method must not be the cause of disease or illness. Indeed, the experimental design was for samples to be collected at the same location in all joints: normal, PL with or without cartilage erosion, and OA groups. This is because if samples were collected at different locations (weight-/non-weight-bearing sites or lesion/normal sites) it would affect the comparison of gene expression among the four groups. In the PL with cartilage erosion group, the expression of genes in cartilage taken from a lesion was similar to that of OA [35]. But our findings have shown than not only do chondrocytes from a lesion demonstrate a similarity to chondrocytes from OA, they also have a marked effect on normal cartilage tissue in the same bone.

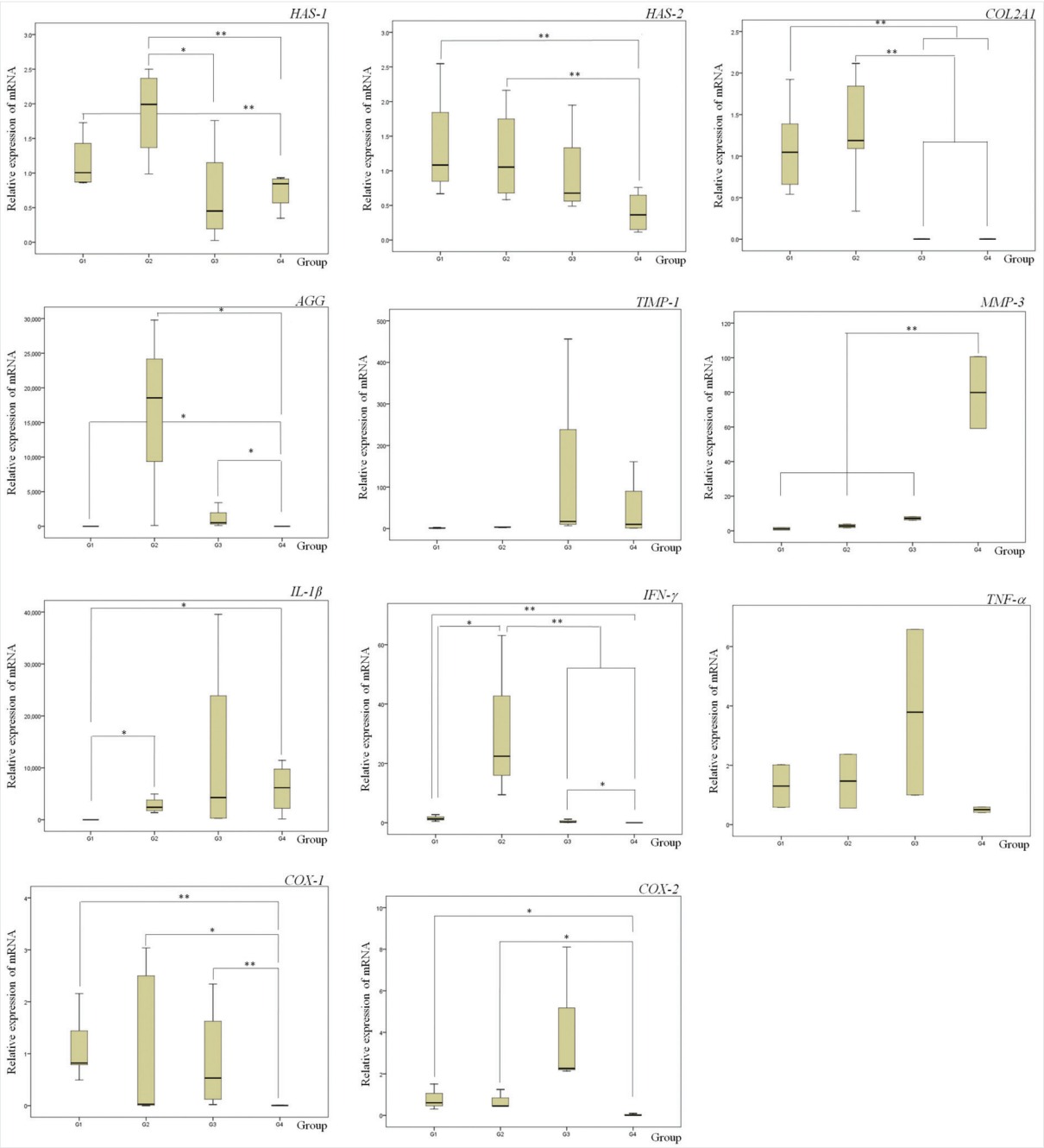

Fig 3. The relative expressions of 11 genes from the articular cartilage, as considered in the four study groups: G1 = normal, G2 = patellar luxation without cartilage erosion, G3 = patellar luxation with cartilage erosion, and G4 = osteoarthritis (*P<0.05; **P<0.01)

Şekil 3. Artikular kartilajda 11 genin orantısal ekspresyonları G1 = normal, G2 = kartilaj erozyonu olmayan patella çıkığı, G3 = kartilaj erozyonlu patella çıkığı ve G4 = osteoartritis (*P<0.05; **P<0.01)

To the best of our knowledge, this research is the first to demonstrate the expression of genes in PL with and without cartilage lesion as having the potential to develop into OA. This study has shown that the expression levels of *AGG* (8,302-fold) and *IFN-γ* (18-fold) from the articular cartilage and *HAS-1* (1,404-fold), *AGG* (19,814-fold), and *IL-1β* (365-fold) from the synovial membrane of PL without cartilage erosion are the highest among all the four groups. The expression of *IL-1β* (9,872-fold) and *COX-2* (48-fold) from the articular cartilage and *TIMP-1* (332-fold) and *MMP-3* (44-fold) from the synovial membrane of PL with cartilage erosion was found to be the highest among all the four groups. In PL with or without cartilage erosion, it was found that the *AGG* and the *IL-1β* expression levels from the articular cartilage and the *HAS-1* expression from the synovial membrane had up-regulated in comparison with the normal and the OA groups.

Among the cytokines involved in the OA process, *IL-1β* and *TNF-α* are found to play important roles as major

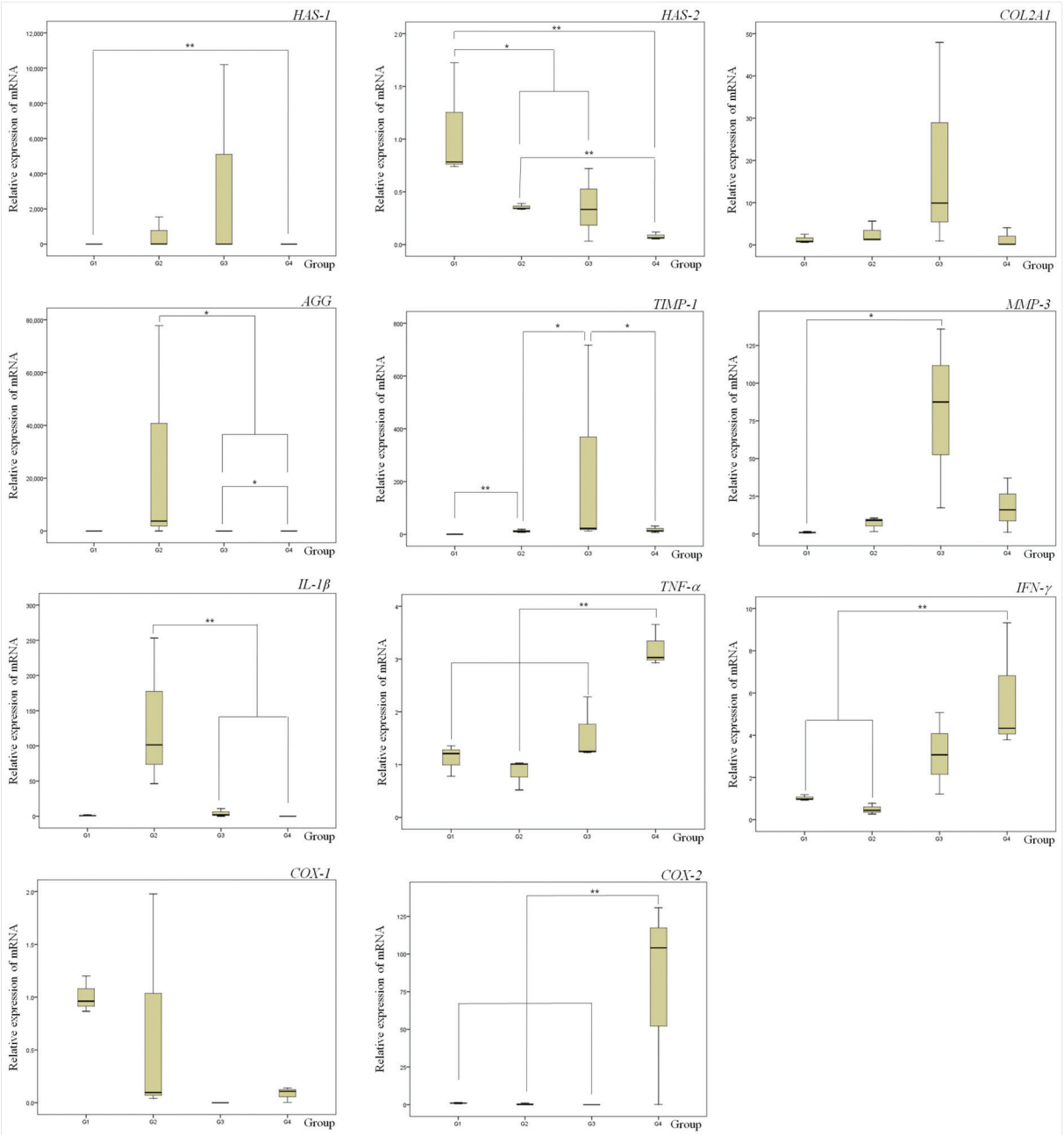

Fig 4. The relative expression of 11 genes from the synovial membrane, as considered in the four study groups: G1 = normal, G2 = patellar luxation without cartilage erosion, G3 = patellar luxation with cartilage erosion, and G4 = osteoarthritis (*P<0.05; **P<0.01)

Şekil 4. Sinoviyal zarda 11 genin orantısal ekspresyonları G1 = normal, G2 = kartilaj erozyonu olmayan patella çıkığı, G3 = kartilaj erozyonlu patella çıkığı ve G4 = osteoartritis (*P<0.05; **P<0.01)

OA-induced cytokines. Our study found that the cartilage and synovial cells of PL expressed *IL-1β* in levels higher than normal and OA. In the articular cartilage of PL with and without cartilage erosion, *IL-1β* was observed to have the highest level of expression in comparison with other genes. But the expression levels from the synovial membrane of both the groups were observed to have slightly up-regulated. From this result, it is possible to conclude that PL with or without cartilage erosion can increase the expression levels of *IL-1β* and *TNF-α*, which can lead to the development of a catabolic pathway to OA. Moreover, *MMP-3* is an enzyme that responds to the catabolic pathway, and the expression of this gene was found to be significantly high in the articular cartilage. The modulated expression of *MMP-3* was found in the articular cartilage and the synovial membrane of PL with cartilage erosion, while the expression from PL without cartilage erosion was observed to be mild. It is possible that cartilage erosion is one cause of *MMP-3* expression, which increases the degradation process in articular joints affected with PL. The enzyme *MMP-3* can cleave collagen, aggrecan, and link protein, while *TIMP*s inhibit the activity

of *MMP*s. This study evaluates the expression of *MMP-3* and *TIMP-1* because these two genes are related, as previously described [36,37]. An increase in the level of the enzyme *MMP-3* in comparison with *TIMP-1* in the cartilage and the synovial membrane would explain the decrease in proteoglycan. In this study, the expression levels of *MMP-3* and *TIMP-1* from the articular cartilage of PL with and without cartilage erosion and OA are together up regulated.

As is well known, *IFN-γ* is a pro-inflammatory cytokine that plays a key role in maintaining immune homeostasis in patients with rheumatoid arthritis (RA) and joint inflammation [8]. This study found that the expression of *IFN-γ* in the articular cartilage from patella luxation without cartilage erosion was up-regulated, but that the expression of *IFN-γ* in the articular cartilage from PL with cartilage erosion and OA were down-regulated; however, they were not found to be very significantly different. This finding is similar to the finding reported by Tsuchida et al.[18], which is that the level of the synovial fluid of the cartilage defect joint is higher than the level of the cartilage of OA. Whereas in the synovial membrane of PL with and without cartilage erosion, it was found that *IFN-γ* showed mild up regulation, significant ($P<0.05$) up regulation was found in the synovial membrane of OA.

One of the multifunctional enzymes involved in the normal and the pathologic pathways is COX, of which two isoforms have been characterized. *COX-1* is expressed constitutively in many organs/tissues in body, while *COX-2* up-regulates in the inflammation pathway. Our study found a low expression of *COX-1* in the cartilage and the synovial membrane of PL with cartilage erosion and OA groups, while *COX-2* expression from the cartilage and the synovial membrane was observed to be the highest in PL with cartilage erosion and OA group. Increasing amounts of *IL-1β* and *TNF*-α were detected in cartilage and synovial membrane samples taken from both the OA and PL with cartilage erosion groups. Both of these cytokines have the ability to upregulate *COX-2* gene expression. *TNF* activates not only the degradation pathway in OA, but also the sensory neurons, which is what induces neuropathic pain [38]; an increase in *TNF-α* in PL and OA can be a cause of pain.

The HAS enzymes are secreted by chondrocyte and synoviocyte, and the three related synthase isoenzymes are *HAS-1*, *HAS-2*, and *HAS-3*. The predominant enzymes are *HAS-1* and *HAS-2*; *HAS-1* is a major HAS isoform produced from synoviocyte, while *HAS-2* is a major isoform produced from cartilage [39]. Our study found the gene expression of *HAS-1* and *HAS-2*, but not *HAS-3* because *HAS-1* and *HAS-2* are active during the process of tissue damage and repair and produce high molecular weight HA, whereas*HAS-3* produces low molecular weight HA [40]. This study found the *HAS-1* expression from the synovial membrane to be extremely high in PL, both with and without cartilage erosion groups, while it was downregulation the OA group in comparison with the control group. *HAS-2* was observed to be upregulated in PL with or without cartilage erosion but down regulated in the OA group.

Both the synovial membrane and the articular cartilage play important roles in controlling OA. But the difference between the cartilage and the synovial membrane lies in the expression of the genes; even for the same gene, there exists differences in the expression between two tissues in normal or OA joint [18]. All the cytokines that are produced from these two tissues influence the OA mechanism. In the early stages of OA, the expression of cytokine from the synovial membrane is found to be associated with the presence of synovial inflammation [41,42]. Our study found down-regulation of the *COX-2* gene from the synovial membrane of patella luxation with and without cartilage erosion, but the highest occurrence of up-regulation was from the synovial membrane of the OA group. Moreover, *COX-2* in the articular cartilage was observed to be up regulated in PL with cartilage erosion, but down-regulated in PL without cartilage erosion and OA.

This study has demonstrated that PL with or without cartilage erosion expresses pro-inflammatory cytokines and enzymes, and that some anabolic biomolecules are up regulated but some are down regulated. The expression was different between articular cartilage and synovial membrane. The expression of genes from PL with cartilage erosion is similar to that of OA. In conclusion, PL with or without presentation of articular cartilage erosion can lead to OA, based on increasing levels of *IL-1β* observed.

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