＜整形外科＞

①3.0T MRIの高精度画像診断技術を用いた遅延相ガドリニウム造影およびT2マッピング
ー荷重位撮像を含めてー

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④別冊整形外科　運動器疾患の画像診断

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はじめに

変形性関節症（osteoarthritis：OA）は，わが国で自覚症状を有する患者数は約 1,000 万人，X 線像上の患者数は約 3,000 万人といわれており，最終的には不可逆的な関節症性変化をもたらす．末期 OA では人工関節置換術などの手術的治療が必要となるため，できるだけ早期に OA を発見し，治療介入することが重要である．

MRI 撮像は OA の早期発見に有効な方法と考えられるが，従来の T2 強調画像やプロトン密度強調画像では，軟骨の形態的な評価は可能であるが，質的な評価は困難であった．近年，発達してきた遅延相ガドリニウム造影 MRI（delayed gadolinium enhanced magnetic resonance imaging of cartilage：dGEMRIC）や T2 マッピング法で軟骨の質的な評価が可能となってきている．われわれは dGEMRIC と荷重をかけた状態での T2 マッピングで変性軟骨の評価を行ったので報告する．

Ⅰ．遅延相軟骨造影 MRI（dGEMRIC）

造影剤である gadolinium diethylene triamine-pentaacetic acid（Gd-DTPA^{2-}）を経静脈的に投与し，撮像する方法である．陰性荷電した Gd-DTPA^{2-}と同じ陰性荷電をもつ軟骨基質の構成成分のグリコサミノグリカン（GAG）が反発する性質を利用している[1,2]（図 1）．述べたように GAG は陰性荷電を有しており，また変性軟骨では GAG 濃度が低下している．そこで Gd-DTPA^{2-}を静脈内投与すると，電気的な反発力により変性軟骨では健常軟骨に比べ Gd-DTPA^{2-}が取り込まれやすい．Gd-DTPA^{2-}は T1 値を短縮させるため，変性軟骨では T1 値が短縮することとなる．つまり，健常軟骨は比較的長い T1 値となるが，変性軟骨では T1 値は健常軟骨に比べて短縮することになる．

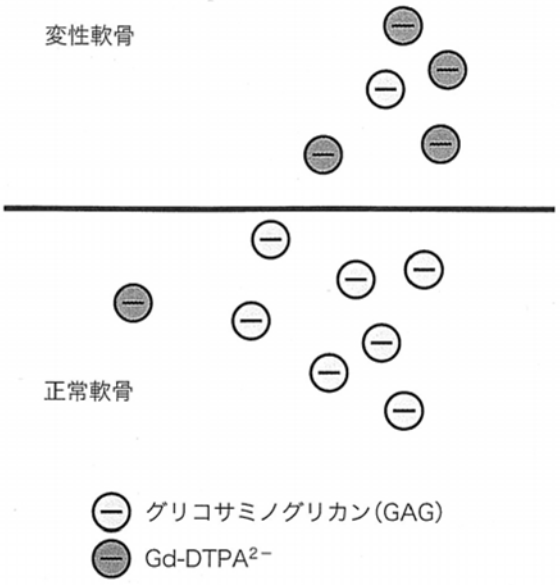

図 1．dGEMRIC の原理（文献 1 より引用）．変性が進行すると GAG 濃度が減少するため，投与した Gd-DTPA^{2-}が軟骨内によく浸透する．結果として T1 値が減少する．

Key words

OA，delayed gadolinium enhanced MRI of cartilage（dGEMRIC），T2 mapping

*Evaluation of the articular cartilage of the knee in delayed gadolinium enhanced magnetic resonance imaging of cartilage（dGEMRIC）and T2 mapping loading magnetic resonance imaging at 3.0 tesla

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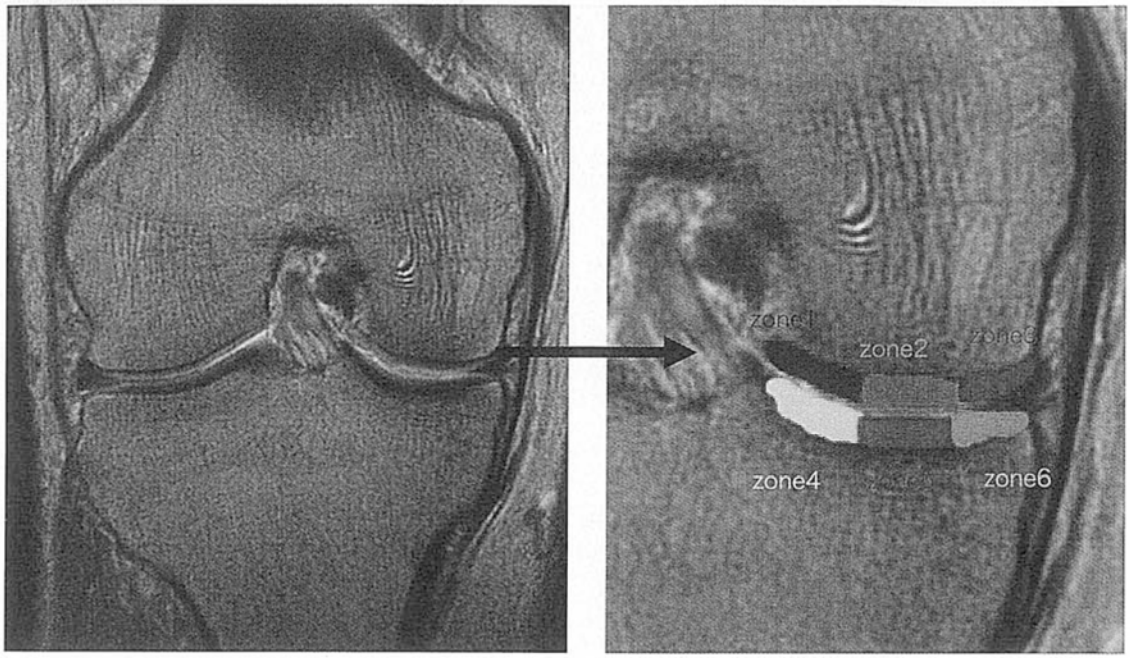


図 2．関心領域（region of interest：ROI）の設定．内側コンパートメントの軟骨面を zone 1 から zone 6 まで冠状断像で三等分し，計 6 つの領域の T1 値を参照する．

dGEMRIC は GAG 濃度をパラメータとしており，初期 OA をとらえるのに有用な方法と考える[3]．しかしながら，造影剤を投与するため投与には慎重になる必要がある．

❶対象および方法

当科に外来通院中の膝 OA 患者 21 例 21 膝を対象とした．男性 4 膝，女性 17 膝であり，年齢は平均 66（42～76）歳，身長は 156（147～165）cm，体重は 60.2（42～97）kg，body mass index（BMI）は 24.7（17.9～41.4）であった．Kellgren-Lawrence 分類（K-L 分類）では grade Ⅰ：2 膝，grade Ⅱ：11 膝，grade Ⅲ：4 膝であった．

MRI は Signa Excite HD（GE Medical Systems 社，Waukesha）を用いて撮像した．撮像条件は，TE 7.2，TR 3,000，TI 1,500，500，300，100，50，ETL（echo train length）7，band width（BW）62.5 kHz，FOV 120 mm，slice thickness 3.0 mm，matrix 288×288，NEX 1，scan time 2：18 とした．

dGEMRIC の撮像方法は，まず MRI の 2 時間前に Gd 造影剤を通常投与量の倍量である 0.2 mM/kg 投与した後に，Gd の軟骨内浸透のために 10 分間程度歩行してもらい，膝関節軟骨の T1 マップを撮像後にプロトンおよび T2 強調画像を撮像した[4]．

図 2 に示すように，内側コンパートメントを 6 つの zone に分け，関心領域を設定し，それぞれの zone の T1 値の平均値を算出した．同一症例を介入時およびその 1 年後に撮像し，各 zone の T1 値を測定し，また各 K-L 分類 grade の T1 値を測定して検討した．

統計には one-way ANOVA を用い，$p<0.05$ を有意差ありとした．

❷結　果

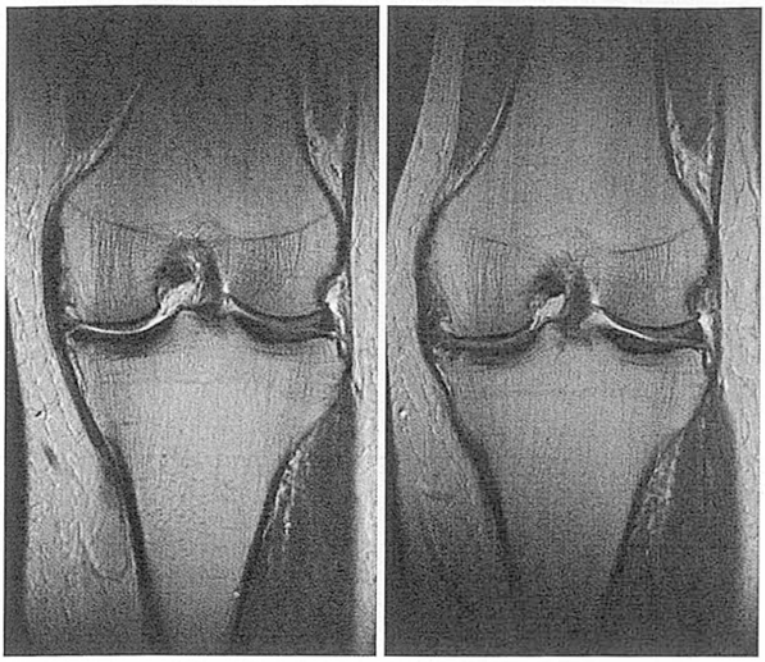
大腿骨側と脛骨側全体での T1 値は，介入時では脛骨側のほうが高値を認めた．いずれも K-L grade が進行するにつれて低下する傾向にあった．介入後 1 年でも，脛骨側のほうが高値を認め，K-L grade が進行するにつれて低下する傾向にあったが，介入時にみられた脛骨内側の K-L grade Ⅰと K-L grade Ⅲの統計学的な有意差は消失していた．

これを各 zone 別に検討すると，介入時では，いずれの zone でも grade が高くなるにつれて T1 値が低下する傾向にあった．介入後 1 年では介入時と同様に，いずれの zone でも grade が高くなるにつれて T1 値が低下する傾向にあったが，各 zone での T1 値は低下しており，介入時にみられた有意差は消失していた．

❸症例提示

症　例．72 歳，女．

単純 X 線像では，K-L 分類 grade Ⅱの内側型膝 OA を認めた．立位大腿脛骨角は 178°，BMI は 21.3 であった．プロトン強調画像では，介入時，介入後 1 年ともに明らかな軟骨面の不整像は認めなかった（図 3）．しかし



a．介入時　　b．介入後 1 年

図 3．症例．プロトン強調画像．軟骨面の不整は認めない．

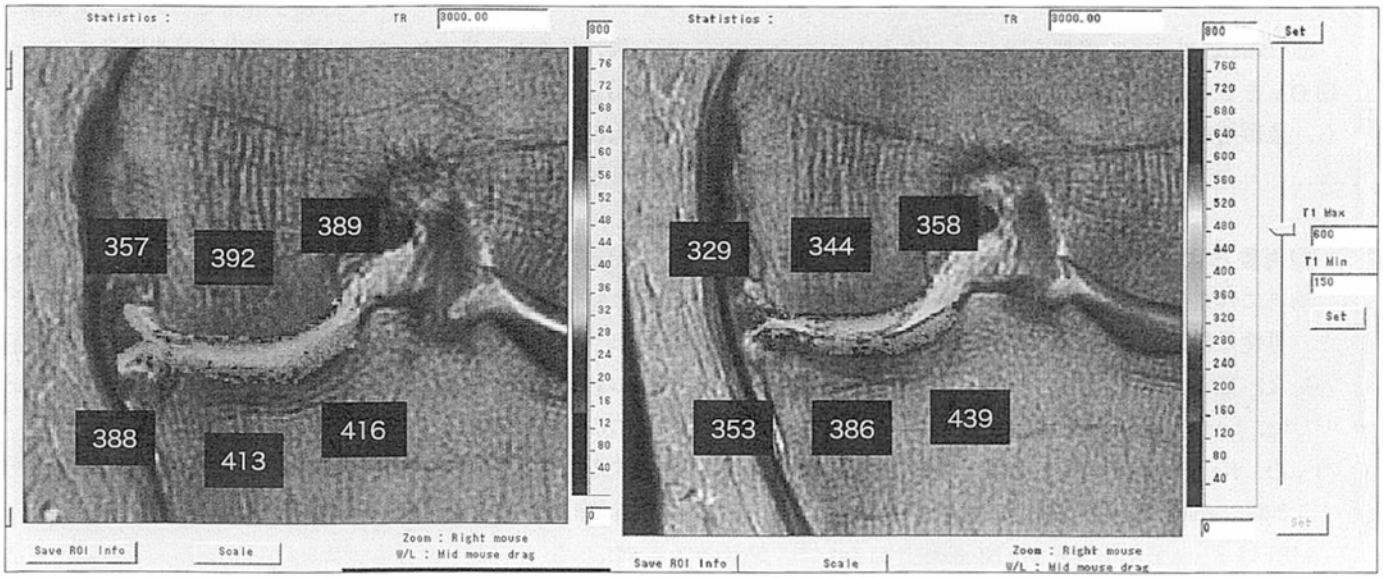


図 4．症例．dGEMRIC 法による T1 値（左：介入時，右：介入後 1 年）．ほぼすべての zone で T1 値は減少しており，より詳細に軟骨変性をとらえている可能性が示唆される．

dGEMRIC により T1 値を測定すると，介入後 1 年になると zone 1，2，3，5，6 で T1 値は減少しており，軟骨変性の進行をより詳細にとらえている可能性が示唆された（図 4）．

Ⅱ．荷重位 T2 マッピング

T2 マッピングは軟骨の構成成分のうち，水分含有量とコラーゲンの配列を反映している．OA にいたると軟骨の変性に伴ってコラーゲンの配列が不整化し，水分含有量が増加するため T2 値は増加する[5]．この T2 値の変化は，dGEMRIC による T1 値の変化よりは変性が進行してからでないと検出されにくいため，より鋭敏に T2 値の変化が反映できるような工夫が必要である．そこで，われわれは荷重下での T2 マッピングを行った．Nishii ら[7]は，22 人の健常人での荷重下 T2 マッピングを行い，荷重をかけることにより T2 値は減少することを報告した．今回，われわれは OA 例と対照群との荷重下での T2 マッピングの

比較・検討を行った．

❶対象および方法

当科に外来通院中の膝 OA 患者 22 例 23 膝を対象とした．男性 9 膝，女性 14 膝であり，年齢は平均 68（55～76）歳，身長は 161（150～178）cm，体重は 59.1（52～70）kg，BMI は 22.8（21.2～24.8）であった．

また，膝痛のない 7 例 7 膝を対照群とした．男性 5 膝，女性 2 膝であり，年齢は平均 34（25～47）歳，身長は 169（158～178）cm，体重は 61.1（45～70）kg，BMI は 21.2（18.0～23.6）であった．MRI は dGEMRIC 撮像と同様に GE Medical Systems 社製の Signa Excite HD を用いた．撮像条件は，2D-FSE（multi spin echo），TR 1,500，TE 13，26.1，39.1，52.2，65.2，78.2，91.3，104.3，ETL 8，BW ±25 kHz，FOV 12.0 mm，slice thickness/spacing 3.0/0 mm，matrix 384×256，coil：GPFlex，plane：sagittal，scan time 6：27 とした．

T2 マッピングの撮像方法であるが，通常の T2 マッピング法による撮像を行った後に，L-spine（DynaWell 社，Billdal）を用いて，体重の 25%が患側にかかるように軸方向へ体重をかけた（図 5）．画像解析ソフトは大阪大学提供の T2/T1/ADC 値解析ソフト Baum 1.15 を用いた．

内側型 OA であるので内側コンパートメントに着目し，大腿骨側・脛骨側を測定し，非荷重時，荷重時それぞれの T2 値を算出し，OA 群および対照群の値を算出した．

❷結　　果

内側コンパートメントにおいて，大腿骨側・脛骨側ともに OA 群，対照群ともに表層は深層に比べて高値であった．一方，荷重をかけると，対照群では T2 値は低下したが，OA 群では不変あるいは軽度上昇した．このことより，変性した軟骨では荷重に対する応答が変化し，より軟骨変性が進行していく可能性が示唆された．

❸症例提示

対照群の症例と OA 群の症例を図 6 に示す．対照群では荷重により荷重部での T2 値が減少したのに対し，OA 群では T2 値はほぼ変化がなかった．

Ⅲ．考　　察

近年，整形外科分野において関節軟骨の構成成分や水分に着目し，軟骨の変性を早期にとらえる定量的 MRI（quantative MR imaging）が盛んに行われている．具体的には今回述べた dGEMRIC や T2 マッピング，その他 T1 rho，拡散強調画像などがあげられる．今回，われわれは

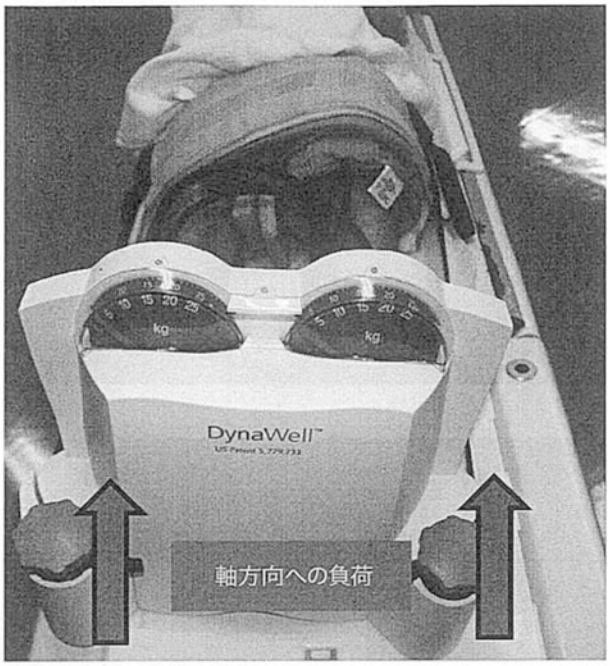


図 5．荷重のための使用機器（DynaWell 社製 L-spine）．
患側へ体重の約 25%を負荷する．

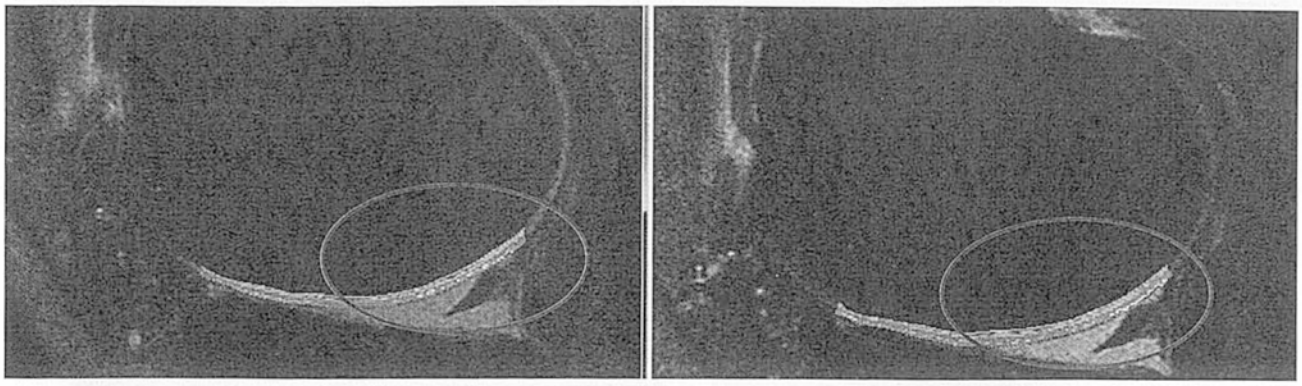
dGEMRIC や T2 マッピングに着目した．

dGEMRIC における今回の検討では，介入時には全体として大腿骨側が脛骨側より低値を示しており，大腿骨と脛骨側に統計学的な有意差を認めた．各 grade での変化量に着目すると，zone 4，zone 6 では，K-L grade が進行するに従って T1 値が統計学的有意差をもって低下していた．これらより OA は脛骨側で進行しやすいということが考えられ，X 線像上でも初期 OA を観察する際にはこれらの部位に着目する必要があることが考えられた．

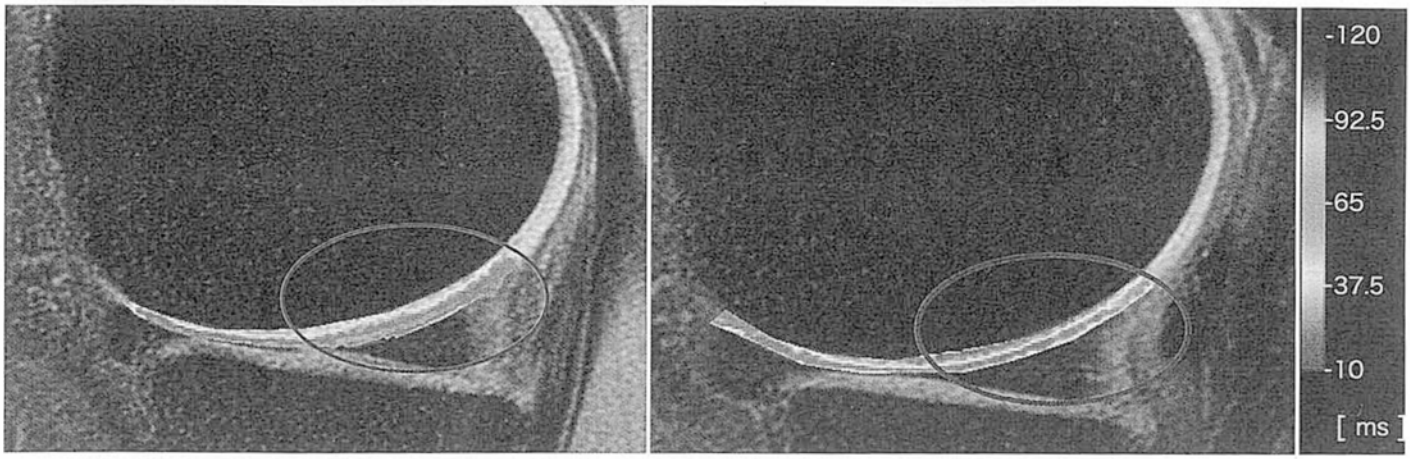
経時的変化では，介入後 1 年では脛骨側で T1 値が低下しており，介入時での統計学的な有意差がなくなっていた．これらより，経時的に関節軟骨でのグリコサミノグリカン濃度が低下しており，軟骨の変性が進行しつつあることが考えられた．

また，T2 マッピングはコラーゲン構築や水分含有量を反映するとされ，dGEMRIC と比較すると初期 OA を把握しにくいとされる．今回，体重の 25%を荷重する T2 マッピング MRI 撮像法により，関節軟骨の性状の変化を画像としてとらえることができた．このことは軽度の荷重，歩行初期などでの疼痛との関連の可能性が示唆され，今後の早期 OA の把握に有用と考えた．

dGEMRIC と T2 マッピングを比較した場合，dGEMRIC のほうが初期の変形性変化をとらえることができると報告されている[6]．しかしながら，dGEMRIC は造影剤の注射を必要とし，検査の費用もかかるなどのデメリットがあり，実際の臨床の現場で頻用される検査にいたっ



a．OA 例



b．対照群

図 6．症例提示（左：非荷重，右：荷重）．T2 値は OA 例ではかわらず，対照群では減少している．

ていない．今回，もともと初期の変形性変化にはあまり鋭敏ではないとされる T2 マッピングに荷重負荷をかけることで，初期 OA の特異的な T2 値の変化をとらえることができた．今後，症例を重ねていくことで，より患者の負担が少なく初期 OA をとらえることができる可能性が示された．

ま と め

1）変形性膝関節症における遅延相軟骨造影 MRI および荷重下 T2 マッピングでの軟骨評価を行った．

2）遅延相軟骨造影 MRI および荷重下 T2 マッピングにより，従来の MRI でわかりにくかった軟骨障害をより詳細にとらえることが可能であった．

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＜整形外科＞

①Changes in microRNA expression in peripheral mononuclear cells according to the progression of osteoarthritis

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Changes in microRNA expression in peripheral mononuclear cells according to the progression of osteoarthritis

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Abstract MicroRNAs (miRNAs) are a family of non-coding RNAs that play an important role in human diseases, including osteoarthritis (OA). The objective of this study was to investigate the expression patterns of miRNAs in the peripheral blood mononuclear cells (PBMCs) of OA patients. PBMCs were isolated from 36 patients with OA, 6 RA patients, and 36 healthy controls. The expression patterns of miR-146a, 155, 181a, and 223 in PBMCs were analyzed using quantitative reverse transcription-polymerase chain reaction (qPCR). We investigated the expression patterns of the miRNAs in OA progression, and their relationships with the parameters of age, body mass index (BMI), the femorotibial angle (FTA), and serum keratan sulfate (KS). The relative expression levels of miR-146a, 155, 181a, and 223 in the OA patients were significantly higher than those found in healthy controls. In the early stages of OA, miR-146a and 223 expressions were significantly higher than they were at later stages. There was a significant correlation between the expression of miR-223 and KS. This study demonstrated that high expression levels of miR-146a, 155, 181a, and 223 in the PBMCs of OA patients might be related to the pathogenesis of OA. This evidence could lead to the elucidation of the mechanism underlying OA pathogenesis and hence to a novel therapeutic strategy for OA.

Keywords MicroRNAs · Osteoarthritis · Mononuclear cell

Abbreviations

OA	Osteoarthritis
K-L	Kellgren-Lawrence
PBMC	Peripheral blood mononuclear cell
BMI	Body mass index
FTA	Femorotibial angle
KS	Keratan sulfate

Introduction

Osteoarthritis (OA) is the most widespread connective tissue disorder that affects the synovial joints [1–4]. Clinical findings of an inflammatory process, such as joint swelling or synovial effusion, are some of the most common clinical features of primary OA. Despite the high prevalence of OA, its etiology has not been elucidated. Many studies have been conducted on the articular cartilage of OA (e.g., chondrocytes, extracellular matrix, and chemokines), and they have suggested that the pathogenesis of primary OA is predominantly caused by biomechanical alterations of the articular cartilage [5–7]. However, OA lesions are not limited to the articular cartilage. They are also seen in bone and synovial tissues. Inflammation in synovial tissues has been well documented, and evidence suggests that synovial tissues may play a crucial role in the pathogenesis and progression of OA. Several studies have demonstrated that mononuclear cells, such as macrophages, B cells, and T cells, which aggregate in OA synovial tissues, contribute to cartilage degradation [8]. A number of studies have suggested that OA might be a systemic disease, like rheumatoid arthritis (RA), rather than only a joint disease.

Recent studies indicate that genetic alterations in non-coding RNA contribute to the pathogenesis of human disease [9]. A new class of small non-coding RNAs called microRNAs (miRNAs) regulates gene expression by binding to target mRNAs' 3′UTR to prevent translation [10–13]. Many miRNAs are evolutionarily conserved across phyla, and several exhibit tissue-specific or developmental stage-specific expression patterns associated with several human diseases, including joint disorders, RA, and OA [14–22].

As for the peripheral blood mononuclear cells (PBMCs) in patients with joint disorders, Pauley et al. [19] have demonstrated that the expression of miRNA (miR)-16, 132, 146, and 155 in PBMCs in RA patients is higher than it is in healthy controls. Fulci et al. [27] reported that miR-223 in naïve CD4 lymphocytes was expressed at a higher level in RA patients than in healthy controls. If one considers OA to be a systemic immune disease in which circulating PBMCs accumulate in OA synovial tissues, subsequently resulting in cartilage degeneration and osteophyte formation [8], this raises the possibility that there may be differences in the expressions of several miRNAs in the PBMCs of subjects with OA compared to the expression in those without the condition.

Four miRNAs—miR-146a, miR-155, miR-181a, and miR-223—are reported to be expressed in immune cells and to regulate immune function and inflammation [17, 19, 23–27]. miR-146a, miR-155, and miR-223 were reported to be highly expressed in the synovium and PBMCs in RA patients compared to healthy controls [17–19, 23].

The purpose of this study was to examine the expression patterns of these miRNAs in OA patients. We analyzed their expression patterns in the PBMCs of OA patients and compared the results with those in healthy controls. We also examined altered miRNA expression in different stages of OA, and we investigated relationships between the expression of the miRNAs and age, body mass index (BMI), the femorotibial angle (FTA), and serum keratan sulfate (KS) level.

Subjects, materials, and methods

This study was approved by the Institutional Review Board of Hiroshima University and was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from the healthy controls, OA patients, and RA patients prior to their participation in this study.

Study participants

Thirty-six patients (7 males, 29 females; mean age 68.0 ± 11.0 years, range 32–83; mean BMI 25.1 ± 4.2, range 19.6–41.4) with OA, 6 RA patients (3 males, 3 females; mean age 67.3 ± 8.0 years, range 59–77), and 36 healthy controls (19 males, 17 females; mean age 32.3 ± 8.5 years, range 21–58) were included in the study (Tables 1, 2, 3). These patients were randomly selected from the patients with knee OA at our special knee outpatient clinic. OA was diagnosed according to the American Rheumatism Association criteria described by Altman et al. [28]. Patients with RA, ankylosing spondylitis, psoriatic arthritis, chronic reactive arthritis, a history of cancer,

Table 1 Demographic data of healthy controls

Subject	Age (years)	Sex	Height (cm)	Weight (kg)	BMI
1	34	M	163	65	24.5
2	32	M	178	65	20.5
3	34	M	168	62	21.9
4	32	M	167	56	20.1
5	32	M	180	67	20.7
6	35	M	165	59	21.7
7	33	M	170	63	21.8
8	32	F	155	40	16.6
9	33	M	174	78	25.8
10	36	M	163	70	26.3
11	33	M	178	65	20.5
12	58	M	180	78	24.0
13	29	F	160	58	22.7
14	28	F	157	46	18.6
15	26	M	171	51	17.4
16	25	F	156	53	21.8
17	25	F	160	53	20.7
18	54	M	165	52	19.1
19	24	M	171	78	26.7
20	24	F	167	56	20.1
21	21	F	158	46	18.4
22	52	F	156	62	25.5
23	43	F	162	58	22.1
24	30	F	157	57	23.1
25	35	M	172	81	27.3
26	33	M	173	78	26.0
27	28	F	158	54	21.6
28	22	F	166	50	18.1
29	26	F	156	56	23.0
30	27	F	163	56	21.1
31	23	F	163	54	20.3
32	39	M	170	78	26.9
33	36	M	173	72	24.1
34	25	F	162	57	21.7
35	31	F	170	64	22.1
36	34	M	172	70	23.7

BMI Body mass index

Table 2 Demographic data of rheumatoid arthritis patients

Patient	Age (years)	Sex	Larsen grade	Medication	RF (mg/dl)	CRP (mg/dl)
1	76	F	1	MTX, NSAIDs	209.3	0.53
2	59	F	1	Salazosulfapyridine	129.2	4.12
3	59	M	5	MTX, prednisone, NSAIDs	49.5	3.53
4	64	M	5	Bucillamine, NSAIDs	65.3	2.57
5	69	M	2	Bucillamne, NSAIDs	22.9	0.31
6	77	F	4	Salazosulfapyridine	160.9	0.16

RF Rheumatoid factor, *CRP* C-reactive protein, *MTX* methotrexate, *NSAIDs* non-steroidal anti-inflammatory drugs

or bilateral knee surgery such as joint replacement were excluded. The OA patients included in this study did not have any serious systemic disease, and no abnormal findings were indicated by general blood examinations. The healthy controls were not obese and did not have inferior limb malalignment, or a history of knee injury or knee disorders. The RA patients, as the disease controls, were diagnosed according to the American Rheumatism Association criteria for RA. Their mean disease duration was 7.5 years, ranging from 2 to 17 years, and all patients were being treated with anti-rheumatoid drugs.

Radiographic evaluation

Bilateral knee radiographs were obtained with antero-posterior and lateral views taken of the symptomatic knee in full extension. An experienced musculoskeletal radiologist and a rheumatologist, both blinded to the clinical data, graded all of the films according to the features of the Kellgren-Lawrence (K-L) classification grade. Where both knees were symptomatic and showed radiographic changes of OA, the knee with the least severe disease was used. Radiographic measurements were calculated from full-length weight-bearing anteroposterior radiographs of the leg, including the hip, knee, and ankle. The femorotibial angle (FTA) was defined as the angle between the femoral and tibial anatomical axes. The FTA was formed by lines drawn from the mid-point between the tibial spines to the center of the talocrural joint and lines drawn from the midpoint of the distal femur shaft to that of the proximal tibia.

Isolation of human PBMCs

We collected peripheral blood from participants and put it, drop by drop, into Dulbecco's phosphate-buffered saline (DPBS; Nacalai Tesque, Kyoto, Japan), containing 0.5 M ethylenediamine tetraacetic acid (EDTA; Invitrogen, Carlsbad, CA, USA) and mixed well. The mixture was slowly loaded into Histopaque® (Sigma-aldrich, St. Louis, MO, USA) and centrifuged at $1000\times g$ at room temperature for 10 min. PBMCs accumulated as the middle white monolayer. After the supernatant was discarded, only the white monolayer cells were aspirated and they were put into the DPBS-EDTA mixture (DPBS-E). They were centrifuged at $400\times g$ at 4°C for 10 min. The supernatant was discarded, and DPBS-E and ammonium chloride (The Cell Experts™, Vancouver, BC, Canada) were added at the proportion of 1:3. The mixture was allowed to stand at room temperature for 10 min. After centrifugation at $400\times g$ at 4°C for 10 min, the supernatant was discarded, and DPBS-E was added and mixed well. This final process was repeated several times. The remaining white cells were PBMCs.

A sandwich 5-D-4 enzyme-linked immunosorbent assay (ELISA) method was used for determining serum KS. The anti-KS monoclonal antibody (5-D-4) and all reagents were available in the KS ELISA kit provided by Seikagaku (Tokyo, Japan) [29].

Samples of the KS standards and diluted samples were added to the wells. Next, the plate was incubated at 37°C for 60 min. After the plates were washed, 25 µl/well of horseradish peroxidase-conjugated streptavidin and 25 µl/well of biotinylated antibody were added to the plate and the plate was incubated at 37°C for 60 min. The plates were washed with the washing buffer, and then 50 µl/well of substrate solution (tetramethylbenzidine) was added to the plate and the plate was incubated at room temperature for 10 min. The reaction was stopped by adding 50 µl 1 N HCL. The absorbance at 450 nm (reference wavelength 630–650 nm) was determined. The KS concentration was determined by applying the absorbance of each sample to the calibration curve.

Quantitative polymerase chain reaction

Total RNA was isolated from PBMCs with Trizol reagent (Invitrogen) for polymerase chain reaction (PCR) analysis. Total RNA yields were calculated and quality was determined using absorption spectrochemical analysis. Quantitative polymerase chain reaction (qPCR) assays were performed using a TaqMan miRNA assay kit (Applied Biosystems, Foster city, CA, USA). We assessed the expression of miR-146a, 155, 181a, and 223. Reverse transcriptase reactions of mature miRNAs contained a sample of total RNA, 50 nM stem-loop RT primer, 10 × RT

Table 3 Demographic data of osteoarthritis patients

Patient	Age (years)	Sex	K-L classification grade	FTA (°)	Height (cm)	Weight (kg)	BMI	Medication	Joint effusion	CRP (mg/dl)
1	74	F	4	148	153	63	26.9	None	Positive	0.07
2	40	F	2	175	164	84	31.2	NSAIDs, HA	Negative	0.1
3	78	F	4	183	150	44	19.6	None	Negative	<0.02
4	83	F	4	183	148	52	23.7	NSAIDs	Negative	0.1
5	65	F	3	177	162	74	28.2	HA	Negative	<0.02
6	70	F	2	172	158	52	20.8	None	Negative	<0.02
7	64	M	3	180	163	62	23.3	None	Negative	<0.02
8	77	F	2	185	145	52	24.7	NSAIDs	Negative	<0.02
9	67	F	2	175	148	55	25.1	NSAIDs	Positive	<0.02
10	71	F	3	182	148	58	26.5	NSAIDs, HA	Negative	0.06
11	75	F	3	180	144	53	25.6	None	Negative	0.04
12	66	F	3	182	156	52	21.4	None	Negative	<0.02
13	76	M	4	188	153	56	23.9	NSAIDs, HA	Positive	<0.02
14	64	M	2	175	163	76	28.6	NSAIDs	Negative	<0.02
15	70	F	4	193	147	55	25.5	NSAIDs, HA	Positive	0.03
16	71	F	3	181	153	49	20.9	None	Negative	<0.02
17	73	F	3	180	160	63	24.6	HA	Negative	<0.2
18	72	F	2	178	159	54	21.4	HA	Negative	0.74
19	32	M	2	177	178	93	29.4	NSAIDs, HA	Positive	0.05
20	71	F	4	182	158	57	22.8	HA	Positive	<0.02
21	60	M	4	191	165	73	26.8	HA	Negative	0.09
22	60	F	2	177	153	97	41.4	NSAIDs, HA	Positive	<0.02
23	72	F	3	180	156	52	21.4	None	Positive	0.38
24	68	F	2	179	160	58	22.7	HA	Negative	<0.02
25	60	F	4	180	156	62	25.5	HA	Negative	0.03
26	51	M	1	179	167	60	21.5	None	Negative	<0.02
27	57	F	2	179	158	54	21.6	HA	Negative	<0.02
28	73	F	3	181	143	48	23.5	None	Positive	<0.02
29	82	F	3	180	150	49	21.8	None	Positive	0.12
30	74	F	4	165	149	60	27.0	NSAIDs	Positive	0.06
31	67	F	4	185	148	67	30.6	NSAIDs, HA	Negative	<0.02
32	82	F	4	195	140	40	20.4	HA	Negative	<0.02
33	73	F	3	181	155	53	22.1	HA	Negative	<0.02
34	56	F	4	182	163	75	28.2	NSAIDs, HA	Negative	<0.02
35	73	M	4	185	164	76	28.3	None	Negative	0.05
36	73	F	4	184	153	63	26.9	NSAIDs, HA	Negative	0.03

K-L Kellgren-Lawrence, *FTA* femorotibial angle, *HA* hyaluronic acid

buffer, 100 mM each dNTP, 50 U/μl MultiScribe reverse transcriptase, and 20 U/μl RNase inhibitor; 15-μl reaction mixtures were incubated in a thermocycler (BioRad, Hercules, CA, USA) for 30 min at 16°C, 30 min at 42°C, 5 min at 85°C, and held at 4°C. Quantitative reverse transcription (RT)-PCR was performed using a Mini Opticon Real-time PCR System (BioRad) in a 10-μl PCR mixture containing 1.33 μl RT product, 2× TaqMan Universal PCR Master Mix, 0.2 μM TaqMan probe, 15 μM forward primer, and 0.7 μM reverse primer. All reaction mixures were incubated in a 48-well plate at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, and 60°C for 1 min (performed in triplicate). The U18 (internal control) was used as a control to normalize differences in total RNA levels in each sample. A threshold cycle (C_T) was observed in the exponential phase of amplification, and quantification of relative expression levels was performed using standard curves for target genes and the endogenous control. Geometric means were used to

calculate the $\Delta\Delta C_T$ (delta–delta C_T) values expressed as $2^{-\Delta\Delta C_T}$. The mean value of each control sample was set at 1, and then this was used to calculate the fold-change of target genes.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's post-hoc analysis was used to compare gene expression levels among three or five groups. Pearson's correlation coefficients were used to explore the relationship between the expression levels of the miRNAs and several parameters, including BMI, age, the FTA, and the concentration of KS. We considered p values of <0.05 to be statistically significant. The Smirnov-Grubb's outlier test was applied for the detection of outliers. All analyses were performed using the Stat View version 5.0 statistical package (Abacus Concepts, Berkeley, CA, USA).

Results

miR-146a, miR-155, miR-181a, and miR-223 were intensely expressed in OA PBMCs compared to their expression in the PBMCs of healthy controls

The average relative expression levels of miR-146a, miR-155, miR-181a, and miR-223 in the PBMCs of OA patients were examined by qPCR. The average relative expression levels of miR-146a, miR-155, miR-181a, and miR-223 were 3.8-, 1.9- 1.4-, and 2.9-fold, respectively, in comparison with levels in the healthy controls. The levels were significantly higher in OA patients than in healthy controls (miR-146a, miR-155, miR-223; $p < 0.01$, miR-181a; $p < 0.05$) (Fig. 1). The miR-146a, miR-155, and miR-223 expression levels in the PBMCs of RA patients were significantly higher than those in healthy controls and OA patients. The expression of miR-181a in the RA PBMCs was also significantly higher than that in the healthy controls.

miR-146a, miR-181a, and miR-223 were highly expressed in PBMCs from patients with early OA

To examine the average relative expression levels of miR-146a, miR-155, miR-181a, and miR-223 in OA PBMCs during OA progression, the samples were divided into four grades according to the K-L classification grade. The expression level of miR-146a in K-L Grades 2, 3, and 4 was significantly higher than that in Grade 0. However, the expression level of miR-146a decreased as the K-L classification grade increased. The miR-155 expression levels in Grade 2, 3, and 4 patients were higher than in that in Grade 0 patients, and the miR-155 expression level increased as the K-L classification grade increased. The expression levels of miR-223 in Grade 2, 3, and 4 patients were significantly higher than that in Grade 0, and its expression level decreased as the K-L classification grade increased. There were no significant differences between miR-181a expression levels in the different grades.

These data indicate that miR-146a and miR-223 are highly expressed in OA PBMCs from patients with a low K-L classification grade, and the expression of miR-155 in the PBMCs of OA patients was up-regulated in the late stage of OA (Fig. 2).

Correlation of miRNA expression with clinicopathological parameters

The clinical characteristics of the patients and healthy controls were used to study potential correlations between miRNA expression and pathological parameters. Firstly we examined the effects of age, sex, BMI, joint effusion, and treatment (such as the use of nonsteroidal anti-inflammatory drugs [NSAIDs] or intra-articular injection of hyaluronic acid) as confounding factors in the expression of the miRNAs. In healthy controls, age and BMI did not affect the expression of the miRNAs. In OA patients, a positive correlation between miR-146a and age ($p = 0.033$, $r = 0.32$) was observed (Table 4). As for sex differences, the expression of miR-223 in female OA patients was significantly higher than that in males, while there was no significant difference between the sexes in healthy controls. There was no significant difference in miRNA expression between joint effusion-positive and -negative OA patients; however, the expression of miR-181a in the patients with medication was significantly lower than that in the patients with no treatment (Table 5).

Next, the relationships between miRNA expression and the FTA and KS were investigated. There was no significant correlation between the miRNA expression and the FTA (Fig. 3). Interestingly, however, the expression of miR-223 had a positive correlation with the concentration of KS ($p = 0.022$, $r = 0.41$). The expressions of miR-146, miR-155, and miR-181a had no association with KS (Fig. 4).

Discussion

Growing evidence suggests that miRNAs play a crucial role in the pathogenesis of human disease [14–27], and several reports have demonstrated that miRNAs participate in the pathogenesis of OA [30, 31]. Yamasaki et al. [21] have demonstrated that miR-146 is highly expressed in low-grade OA cartilage, and that its expression is induced

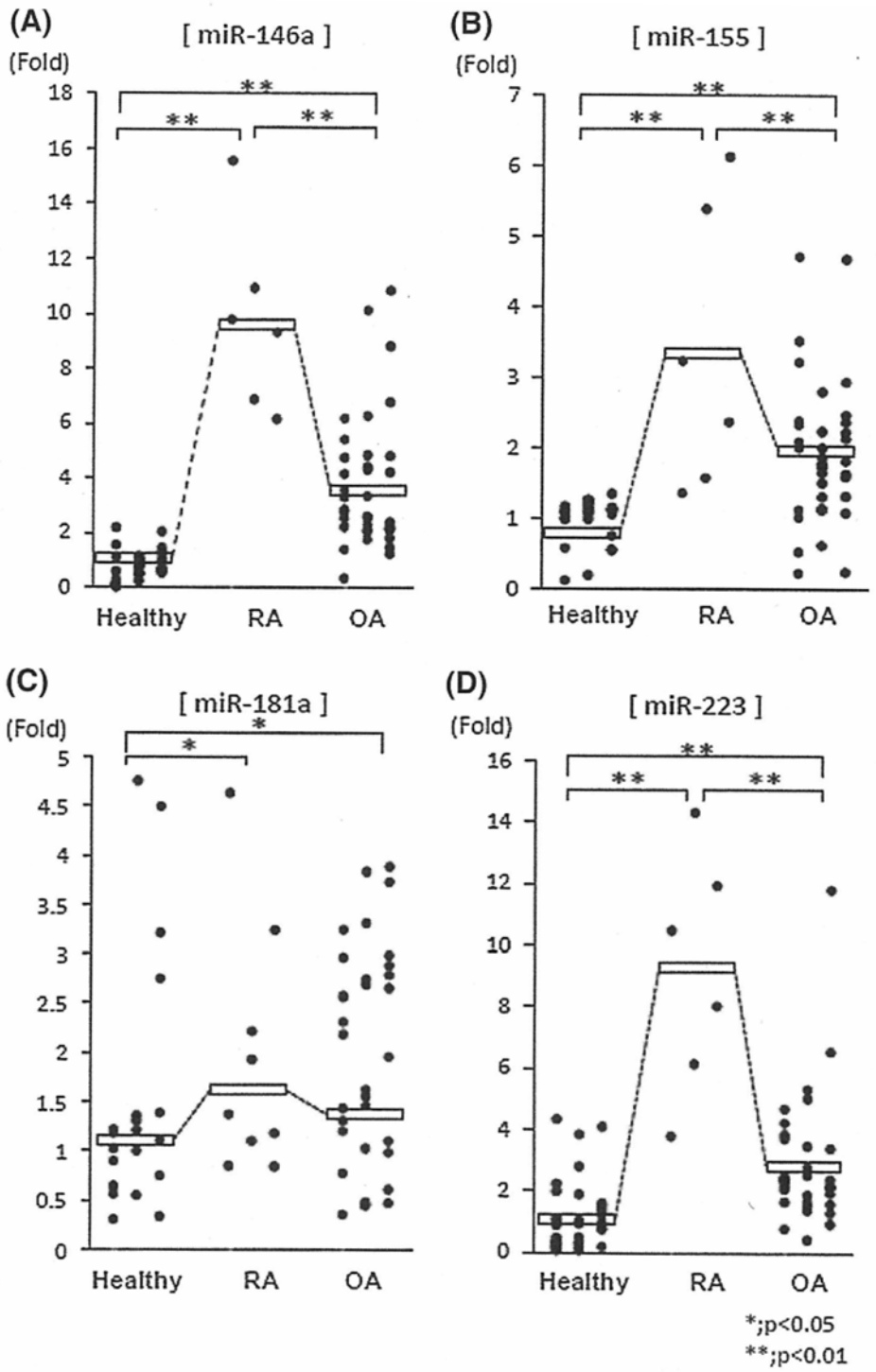

Fig. 1 Expression of microRNAs (miRNAs) in peripheral blood mononuclear cells (PBMCs) of patients with osteoarthritis (*OA*), rheumatoid arthritis (*RA*) patients, and healthy subjects. **a** miR-146a expression, **b** miR-155 expression, **c** miR-181a expression, **d** miR-223 expression. The average relative expression levels of miR-146a, miR-155, miR-181a, and miR-223 were significantly higher for OA patients than for healthy controls based on quantitative real-time polymerase chain reaction (PCR) analysis. *White horizontal bars* indicate mean values. ($*p < 0.05$, $**p < 0.01$)

by interleukin (IL)-1β stimulation. Miyaki et al. [22] reported that a reduction in miR-140 expression in OA cartilage may contribute to the abnormal gene expression pattern characteristic of OA, and miR-140$^{-/-}$ mice showed age-related OA-like changes, characterized by proteoglycan loss and fibrillation of articular cartilage. These findings suggest that miRNAs play a role in the cartilage of OA.

Synovitis is implicated in the pathogenesis of OA. Accumulations of cells such as macrophages and T cells in the synovium of OA patients produce pro-inflammatory cytokines and degradative enzymes, leading to subsequent OA progression. Circulating PBMCs accumulate in the OA synovium; therefore, in the present study, we investigated the expression of miRNAs in the PBMCs of OA patients [8]. Our results may help in the development of a novel

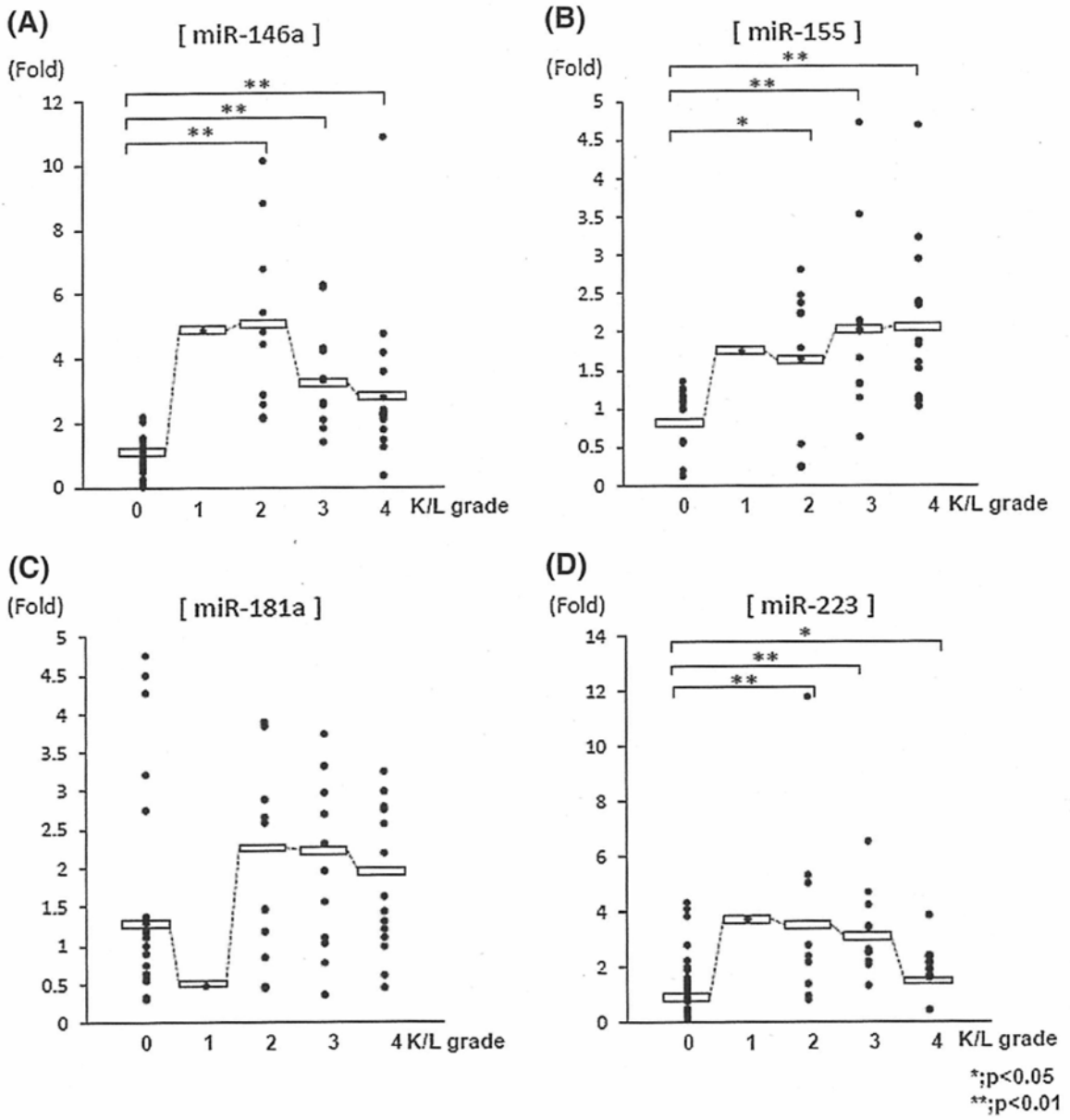

Fig. 2 Expression of miRNAs in PBMCs at each stage of the Kellgren-Lawrence (*K/L*) classification. **a** miR-146a expression, **b** miR-155 expression, **c** miR-181a expression, and **d** miR-223 expression. OA patients were divided into four grades according to the the K/L classification. miR-146a and miR-223 were highly expressed in OA PBMCs from patients with a low K-L classification grade. The expression of miR-155 was significantly higher in late-stage OA compared with earlier stages. *White horizontal bars* indicate mean values. ($*p < 0.05$, $**p < 0.01$)

treatment for OA patients, and recent reports have shown that the methods used for regulating endogenous miRNA expression by the administration of antisense agents or agents that mimic miRNA for treatment in vivo have been improving [32–34]. Moreover, miRNA has been recognized as a useful biomarker to diagnose several diseases, including cancer [35].

Our results show that the expression levels of miR-146, miR-155, miR-181a, and miR-223 in OA patients are significantly higher than they are in healthy controls. Moreover, miR-146 and miR-223 are highly expressed in early-stage OA, with expression gradually decreasing as OA progresses. In contrast, miR-155 was up-regulated in late-stage OA. These outcomes indicate that miRNAs are highly expressed in the PBMCs of early-stage OA patients, and might play an important role in the active stages of OA and its progression. Synovitis in the early stage of OA is of importance for disease progression because of the related production of proinflammatory cytokines from macrophages and T cells present in the synovium.

Table 4 Correlations between microRNA expression and age, and between microRNA expression and body mass index (BMI) in patients with osteoarthritis (OA)

	Age		BMI	
	Healthy	OA	Healthy	OA
miR-146a				
r	0.0072	0.32	0.37	0.099
p	0.49	0.033*	0.06	0.28
miR-155				
r	0.0012	0.083	0.16	0.061
p	0.5	0.32	0.25	0.36
miR-181a				
r	0.38	0.28	0.38	0.078
p	0.26	0.051	0.39	0.33
miR-223				
r	0.13	0.02	0.16	0.12
p	0.29	0.46	0.25	0.24

* In OA patients, miR-146a was positively correlated with age ($p = 0.033$, $r = 0.32$), but BMI did not affect the expression of the miRNAs. In healthy controls, age and BMI did not affect the expression of the miRNAs

Table 5 Correlation between microRNA expression and OA patients' profiles

	Sex			Joint effusion			Medication		
	Male ($n = 7$)	Female ($n = 29$)	*p* value	Positive ($n = 11$)	Negative ($n = 25$)	*p* value	NSAIDs, HA ($n = 24$)	None ($n = 12$)	*p* value
miR-146a	4.7 ± 3.1	4.9 ± 2.1	0.19	4.9 ± 2.1	4.9 ± 2.4	0.47	5.0 ± 2.5	4.7 ± 2.0	0.35
miR-155	2.3 ± 1.7	2.5 ± 1.7	0.33	1.9 ± 1.4	2.7 ± 1.8	0.06	2.6 ± 1.9	2.1 ± 1.0	0.19
miR-181a	1.6 ± 1.2	2.0 ± 1.2	0.13	1.7 ± 1.2	1.7 ± 1.2	0.25	2.2 ± 1.1	1.3 ± 0.9	0.03*
miR-223	1.5 ± 0.7	3.1 ± 2.2	0.02*	3.4 ± 3.1	3.4 ± 3.1	0.33	2.8 ± 2.3	2.9 ± 1.6	0.27

In regard to sex differences, the expression of miR-223 in female OA patients was significantly higher than that in males, while there were no significant differences in miRNA expression between joint effusion-positive and -negative patients. However, the expression of miR-181a in the patients with medication was significantly lower than that in the patients with no treatment

miR-146 is induced in response to proinflammatory mediators, and its induction is regulated by nuclear factor-kappa B [23]. miR-146a was also reported to be expressed in macrophages and T cells in the synovium; therefore, miR-146a expressed in the circulating PBMCs of OA patients might contribute to the pathogenesis of OA due to accumulation in the synovium in the early stage of OA. Pauley et al. [19] demonstrated that miR-146 was highly expressed in RA PBMCs, suggesting that early-stage OA might have a pathogenic process similar to that in RA.

miR-155 is also expressed in RA synovium, and the overexpression of miR-155 in RA synovial fibroblasts has been shown to reduce the expression of matrix metalloproteinase-3 (MMP-3) and impair the induction of MMP-1 and MMP-3 with toll-like receptor ligands and cytokines. Our present results demonstrated that miR-155 expression in the PBMCs of OA patients was higher than it was in the healthy controls, and high miR-155 expression was exhibited in late-stage OA. The high expression of miR-155 in OA PBMCs might reflect reduced MMP-3 expression, which leads to OA progression.

miR-223 is expressed in granulocytes, including macrophages and neutrophils, which secrete matrix proteases and inflammatory cytokines [25, 26, 36]. In our study, we observed that miR-223 expression was significantly correlated with keratan sulfate (KS), which is derived from the degradation of articular cartilage. We confirmed that miR-223 in the OA synovium was more intensely expressed than in healthy synovium (data not shown). miR-223 is also considered to be an important factor for osteoclastogenesis. These results suggest that PBMCs expressing miR-223 accumulate in the OA synovium and likely participate in cartilage destruction, especially during the early stage of OA.

miR-181 is highly expressed in the spleen and thymus, and miR-181 modulates T-cell and B-cell development when ectopically expressed in hematopoietic stem cells [25]. The ectopic expression of miR-181 in murine

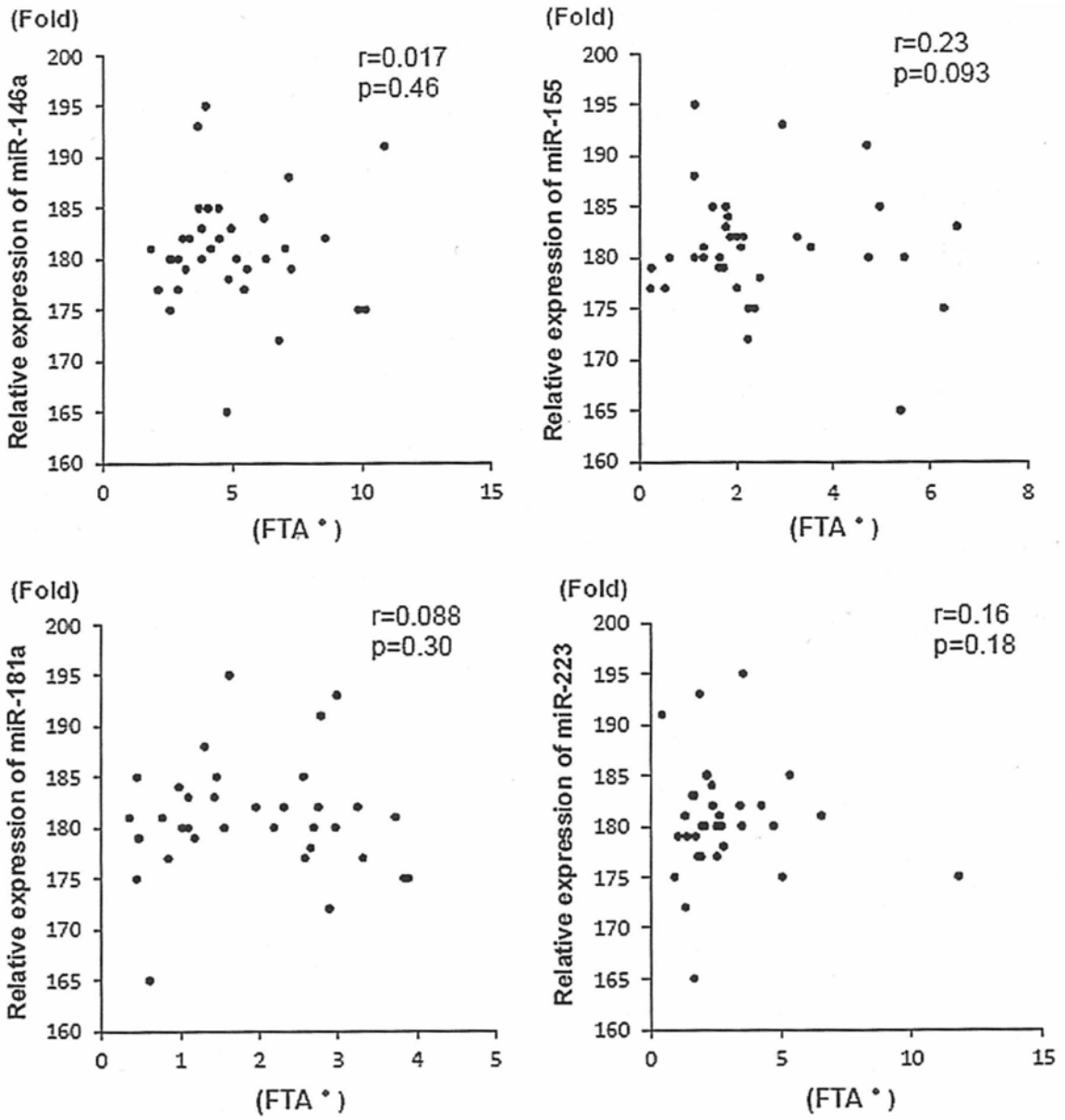

Fig. 3 Correlation between microRNA expression and the femorotibial angle (*FTA*). There was no significant correlation between miRNA expression and the FTA

hematopoietic progenitors results in a significant increase of the B-cell lineage fraction, underscoring possible miRNA involvement in lymphopoiesis. B cells are also reported to play a role in the progression of OA; therefore, there is the possibility that high expression of miR-181a might reflect OA progression [37].

In OA patients, the PBMC expression levels of the miRNAs that we tested were similar to those in RA patients. Two of our OA patients exhibited high expression of miR-146a, miR-181a, and miR-223. Their ages were 40 and 67 years, and they both had a Kellgren-Lawrence (K-L) classification of Grade 2. They both complained of severe joint pain. In addition, two other patients exhibited high expression of miR-146a, another two exhibited high expression of miR-155, and one patient had a high expression level of miR-181a. The mean K-L grade of these five patients was 2.7, and all five patients had severe pain. Therefore, it seems that PBMCs might strongly participate in the pathogenesis of OA, especially during the early stage of the disease.

In the present study, a positive correlation was observed between miR-146a and age. It is reported that serum concentrations of inflammatory cytokines are higher among older than younger OA patients; therefore, it is possible that a high expression of miR-146a (which plays a role in

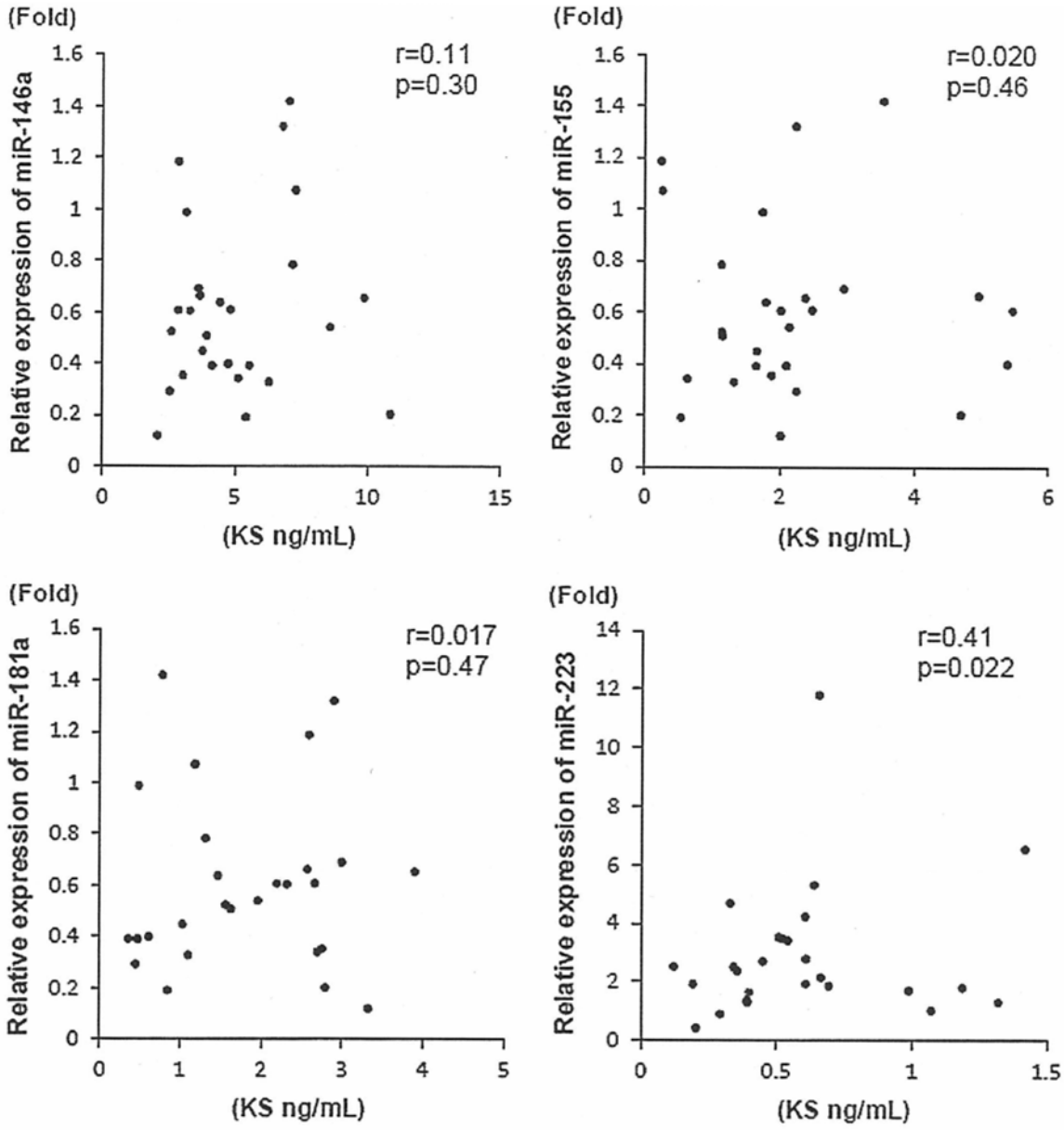

Fig. 4 Correlation between microRNA expression and the concentration of keratan sulfate (*KS*). miR-223 was positively correlated with the concentration of KS as analyzed by Pearson's correlation index ($p = 0.022$, $r = 0.41$)

the inflammatory response) might reflect a high concentration of inflammatory cytokines. High levels of inflammatory markers have been reported to be associated with lower physical function, and this association may lead to a positive correlation between miR-146a and age [38]. In our results, the expression of miR-223 was significantly lower in female than in male OA patients. Women have a higher rate of incidence of OA than men; therefore, it is possible that miR-223 might be involved in the high incidence of OA in women [39]. Further examination will be needed to elucidate the mechanism whereby these miRNAs are involved in the pathogenesis of OA.

Conclusion

The present study demonstrated high expression levels of miR-146a, miR-155, miR-181a, and miR-223 in the PBMCs of OA patients, and indicated that these high levels might be related to the pathogenesis of OA. This evidence could lead to the elucidation of OA pathogenesis and a novel therapeutic strategy for OA.

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knee orthosis of the Japan Orthopaedic Association established for collecting the blood samples of OA patients.

Conflict of interest None.

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＜整形外科＞

①外傷性胸鎖関節後方脱臼の3例

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外傷性胸鎖関節後方脱臼の3例

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今回我々は稀な外傷である胸鎖関節後方脱臼の3例を経験したので，若干の文献的考察を加えて報告する．

症　　例

症例1.

14歳男性，バスケットボール練習中，相手の頭が左胸に当たり受傷した．近医にて経過をみるも軽快しなかったため，受傷後14日に当科受診となった．受診時，左胸鎖関節部の腫脹，陥凹，疼痛，左肩関節の可動域制限を認めた．単純X線およびCT像にて左鎖骨近位部の後方転位を認め（図1，2），左胸鎖関節後方脱臼と診断し全身麻酔下に手術を施行した．徒手整復は不能であったため観血的に整復した．鎖骨近位部の骨端軟骨の離開が認められ，左鎖骨内側骨端離開と診断した．胸骨と鎖骨に骨孔を作成してfiberwireを用いて8の字縫合し手術を終了した．術後6週で骨癒合が得られ，スポーツ活動に復帰した．

症例2.

15歳男性，サッカー試合中に右肩より転倒して受傷した．近医受診し経過観察するも症状改善しないため，受傷後13日目に当科を紹介され受診した．受診時，右鎖骨前面の隆起は消失していた．単純X線像，CTにて右鎖骨内側端の胸骨背側への転位を認め（図1，2），受傷15日目に手術を施行した．術中所見にて鎖骨近位端骨端軟骨が胸骨側に残存していたため，右鎖骨内側骨端離開と診断した．整復後，切開した骨端軟骨を縫合することで良好な固定性が得られ内固定は行わなかった．術後クラビクルバンドにて4週固定，術後6週CTにて骨癒合が得られ，疼痛および右肩可動域制限なく，スポーツ活動に復帰した．

症例3.

25歳男性，柔道の練習中に右肩から落下して受傷した．近医受診し，右肩鎖関節脱臼と診断されたが疼痛持続するため，受傷翌日に当科を紹介され受診した．受診時，右胸鎖関節に腫脹と圧痛を認めた．神経血管障害は認めなかったが，軽度の嚥下困難を認めた．単純X線およびCTにて胸鎖関節後方脱臼を認め（図1，3），同日，全身麻酔下に整復術を施行した．経皮的に骨把持鉗子にて鎖骨を把持し外側上方に牽引することで整復され，良好な固定性が得られた．術後3週間クラビクルバンドにて固定し，術後10週，右肩可動域制限および疼痛なくスポーツに復帰した．

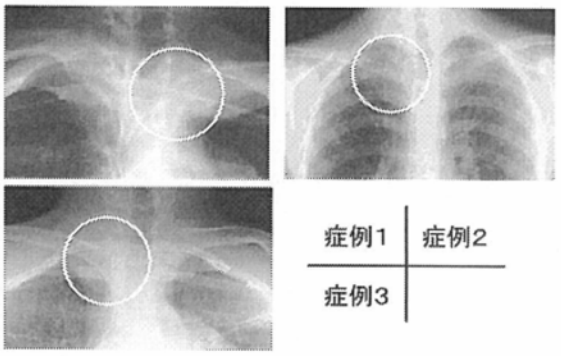

図1　受診時単純X線（Rockwood撮影）

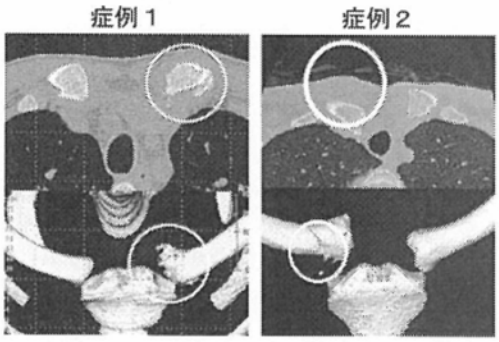

図2　受診時単純CT像（症例1，2）

症例3

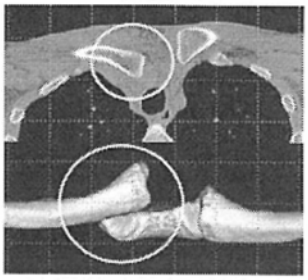

図3　受診時単純CT像（症例3）

考　　察

胸鎖関節後方脱臼は稀な外傷であり，Selesnickらは肩甲帯部の外傷1603例を報告し，胸鎖関節脱臼は10例（0.6%）で，後方脱臼はうち1例のみであったと報告している[1]．

鎖骨近位端の骨化は人体の骨格中でもっとも遅く，15～18歳で始まり，22～25歳で骨端線が閉じるといわれている．22歳頃までの骨端線閉鎖前の症例では，鎖骨近位端よりも脆弱な骨端線に外力が作用して骨端線離開が生じる可能性が高い[2]．

症状は圧痛，腫脹，鎖骨近位端の内側端隆起の消失および肩関節の運動制限，運動時痛があり，また，縦隔圧迫症状として，呼吸困難，嚥下時痛，嚥下困難，神経血管損傷，気管損傷，胸管損傷などが報告されている．縦隔には腕頭動脈，左総頚動脈，左鎖骨下動脈，左右鎖骨下静脈，食道，気管があり，後方に転位した鎖骨によりこれらが損傷を受けると致命的な転帰をとることがある[2]．今回の症例3でも軽度の嚥下困難を認めたが，整復後症状は改善した．

診断には臨床症状に加えて，単純X線所見，CTが用いられる．胸鎖関節の一般のX線撮影では見逃されやすく，Rockwoodによる40°cephalic tilt viewが有効である[2]．また，CTではより正確な診断に加え，縦隔臓器との関係などの情報が得られるため合併症の診断の面からも有効であり，胸鎖関節後方脱臼を疑った際には必須の検査といえる[3]．

治療法として全身麻酔下の徒手整復，経皮的整復，これらが困難な症例では観血的整復術が必要である．受傷後48時間以降の症例では徒手的整復は困難であるとの報告が多い[1]．観血的整復固定法には様々な報告がなされており，関節包，靭帯の縫合，縫合糸による固定，硬線による固定，筋膜，腱を用いた再建術などがある．

今回経験した3例中2例では，早期にX線Rockwood撮影およびCTが受傷直後に施行されておらず早期診断ができなかったため，観血的整復を余儀なくされた．しかし，受傷1日目に受診した症例では早期に診断がつき，非観血的整復術が可能であった．これにより，診断の重要性が改めて確認された．

ま　と　め

1. 稀な外傷である外傷性胸鎖関節後方脱臼の3例を経験したので報告した．
2. 低侵襲な治療のためより早期での診断が重要であることが確認された．
3. 早期診断に際しては，XP Rockwood撮影，CTが非常に有用であった．

文　　献

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