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The factors associated with pain severity in patients with knee osteoarthritis vary according to the radiographic disease severity: a cross-sectional study

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SUMMARY

Objectives: Knee osteoarthritis (OA) pain is suggested to be associated with inflammation and detrimental mechanical loading across the joint. In this cross-sectional study, we simultaneously examined the inflammation and alignment of the lower limb and examined how the pain components varied depending on the disease progression.

Design: One-hundred sixty female medial type of early- [$n = 74$ in Kellgren–Lawrence (K/L) 2] to advanced-stage ($n = 96$ in K/L >2) knee OA subjects (70.5 years on average) were enrolled. Knee pain was evaluated using a pain visual analog scale (VAS) and the pain-related subcategory of the Japanese Knee Osteoarthritis Measure (JKOM-pain). The serum interleukin (sIL)-6 level reflecting synovitis, and the high sensitivity C-reactive protein (hs-CRP) level were measured to evaluate the severity of inflammation. The anatomical axis angle (AAA) was measured as an alignment index. The β-coefficient was estimated after adjusting for age and the body mass index (BMI) using a multiple linear regression analysis.

Results: Multiple linear regression analyses showed that the sIL-6 levels, but not AAA, associated with the pain VAS [$\beta = 10.77$ (95% confidence interval (CI): 4.14–17.40), $P < 0.01$] and JKOM-pain scores [$\beta = 3.19$ (95% CI: 1.93–4.44), $P < 0.001$] in the early stage. Conversely, AAA, but not the sIL-6 levels, was found to be associated with the pain VAS [$\beta = -1.29$ (95% CI: -2.51 to -0.08), $P < 0.05$] and JKOM-pain scores [$\beta = -0.49$ (95% CI: -0.82 to -0.16), $P < 0.01$] in the advanced stage.

Conclusions: The presence of a higher level of sIL-6 and the varus alignment of the joint is associated with pain in early- and advanced-stage knee OA patients, respectively.

Introduction

Pain is the most prominent and disabling symptom of osteoarthritis (OA). Symptom-modifying therapy is the only available treatment for knee OA; therefore, it is important to understand the factors causing the pain in order to optimally treat this common disease. The pain in knee OA is a type of nociceptive pain[1]. It has been speculated that detrimental mechanical loading across the joint and inflammation, especially synovitis, may be the main factors associated with the severity of pain[1,2].

Detrimental mechanical loading across the knee joint is speculated to be one of the main factors in the pathophysiology of knee OA. The alignment of the lower limb has been reported to associate with pain in knee OA[3]. The malalignment of the lower limb and excess body mass have both been considered to be risk factors for the progression of knee OA, due to the association between these factors and the joint load[4–8].

Inflammation is also well known to be associated with the pathophysiology of knee OA. Synovitis in OA may be a secondary phenomenon related to the cartilage and bone alterations induced by the release of degenerative compounds from the extracellular

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matrix of articular cartilage into the synovial fluid[9]. This could further stimulate cartilage damage. Recently, the role of synovitis in OA has attracted particular attention, as synovitis has been revealed to be one of the potential indicators for knee pain and predictive factors for both structural and symptomatic progression of the disease[10–14].

Interleukin (IL)-6 is a proinflammatory cytokine that is produced in the synovium under conditions of synovitis. The serum levels of IL-6 in patients with rheumatoid arthritis (RA) were demonstrated to be related to both the symptoms and progression of the disease, and the inhibition of IL-6 improved the clinical outcomes in patients with RA[15]. IL-6 was also produced by the synovial membrane in patients with knee OA[16], and the serum levels of IL-6 were associated with the change in knee pain over 5 years[17] and with the future prevalence of radiological knee OA[18]. The serum levels of high sensitivity C-reactive protein (hs-CRP) were correlated with inflammatory cell infiltration in the synovial membrane and the levels of IL-6 in the synovial fluid[19]. The hs-CRP level was also reported to be a predictor of knee pain[17]. The serum levels of both IL-6 and hs-CRP were considered to be predictors of a decreased articular cartilage volume in patients with knee OA[19]. Therefore, IL-6, which is associated with synovitis, may also be involved in the pathogenesis of pain associated with knee OA.

While the detrimental mechanical loading across the joint and the synovitis are both speculated to be involved in the pain severity in knee OA, it still remained unclear how the pain components varied based on the progression of the disease. We hypothesized that the factors associated with pain in knee OA varied according to the disease progression. To verify this hypothesis, we divided the patients into two groups according to the radiographic severity of knee OA and investigated the pain severity, the serum IL-6 and hs-CRP levels and the alignment of the lower limb in patients with knee OA, and examined whether the factors associated with pain in knee OA varied according to the radiographic disease severity.

Methods

Subjects and methods

This study was approved by the ethics committee of our university. Patients who first visited the outpatient clinic of Juntendo Tokyo Koto Geriatric Medical Center to seek therapy for knee pain due to OA were asked to participate in the study. All patients who agreed to participate provided their written informed consent before enrollment in this study. All subjects were postmenopausal females with medial knee OA who underwent the initial medical examination at our outpatient clinic between October 2009 and December 2011. The sample size of this study was determined based on the number of patients who demonstrated all these conditions during the study period. The diagnosis of knee OA was established according to the American College of Rheumatology criteria[20]. The inclusion criteria for the present study were (1) subjects who were able to walk with or without walking aids and fulfilled the criteria for knee OA of the medial femoro-tibial joint, (2) subjects who were at least 50 years old, but less than 80 years old, and (3) all subjects had radiographic knee OA with Kellgren–Lawrence (K/L) grade 2 or more as evaluated by the weight-bearing antero-posterior X-rays of the tibio-femoral joint using the bilateral standing extended view[21,22]. The exclusion criteria included (1) patients who had received drugs for knee OA [oral (e.g., non-steroidal anti-inflammatory drugs (NSAIDs) and opioid) or intra-articular injection (e.g., hyaluronic acid and corticosteroids)] prescribed by physicians in the previous 3 months, (2) those who had RA or arthritis due to infection or injury, (3) patients who had undergone joint replacement surgery, (4) patients who had

secondary knee OA, (5) patients with patello-femoral OA with a K/L grade of 3 or higher and (6) patients with severe OA (K/L grade 3 or higher) in their hip joint.

Radiographic evaluation of the stage of progression and alignment of the lower extremity

The staging of knee OA on radiograph was assessed using the K/L grade (scale 1–4)[21]. All radiographs were taken by experienced technicians and were quantified by two readers who were blinded to the clinical information of the patient (HK and YS). Both intra- and inter-observer reproducibility rates were good [interclass correlation coefficient (ICC): 0.97 (95% confidence interval (CI): 0.70–0.97) and 0.95 (95% CI: 0.93–0.98), respectively]. For the statistical analysis, the patients were divided into two groups according to the radiographic severity of knee OA, in line with the methodology of previous reports[17,23]: including an early-stage group (K/L grade of 2) and an advanced-stage group (K/L grade of 3 and 4) group.

The alignment angle was measured by the method reported previously[24–26]. The femoral anatomic axis was found by drawing a line from the center of the tibial spines to a point 10 cm above the tibial spines, midway between the medial and lateral femoral surfaces. For the tibial anatomic axis, a line was drawn from the center of the tibial spines to a point 10 cm below the tibial spines, midway between the medial and lateral tibial surfaces. The internal angles between the femoral and tibial axes were measured using a computer and the angle was designated as the alignment angle (anatomical alignment angle − AAA). In this method, AAA under 180 indicates varus knee alignment.

Knee OA pain

Pain was evaluated by a visual analog scale (VAS, 0–100) and the Japanese Knee Osteoarthritis Measure (JKOM) score[27]. The JKOM is a patient-based, self-answered evaluation score that includes four subcategories: pain and stiffness (JKOM-pain; total of eight questions, 0–32 points), activities of daily living (ADL) score (total of 10 questions, 0–40 points), participation in social activities score (total of five questions, 0–20 points), and general health conditions (total of two questions, 0–8 points) with 100 points as the maximum score. The JKOM score is higher in patients with more pain and physical disability, and this evaluation modality is considered to have sufficient reliability and validity for studies of the clinical outcomes of Japanese subjects with knee OA. The measure has also been shown to have reliability and validity by means of statistical evaluations and comparison with other health-related scales, the Western Ontario and McMaster Universities Arthritis Index (WOMAC) and the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) in our previous study[27]. We assessed the pain severity using both the pain VAS score and JKOM-pain scores in this study.

Serum IL-6 and hs-CRP levels

Serum samples were obtained from all patients on the day that pain and function were assessed, and radiographs were taken. The non-fasting morning blood samples were collected from 9 to 11 in the morning at the outpatient clinic. The serum samples were stored at −80°C until they were analyzed. A chemiluminescent enzyme immunoassay was used for the measurement of the serum IL-6 concentration (minimum 0.2 pg/mL; reagent: human IL-6; cartridge and instrumentation: Lumipulse Forte; Fujirebio, Tokyo, Japan: intra-assay and interassay variations less than 5.2% and 6.5%, respectively). The serum hs-CRP concentration was measured by latex agglutination nephelometry (reagent: CardioPhase hs-CRP; instrumentation: BN

Pro Spec; Siemens Healthcare Diagnostics Inc., NY, USA: intra-assay and interassay variations less than 2% and 3.5%, respectively).

Statistical analysis

Descriptive statistics of age, body mass index (BMI), IL-6, hs-CRP and AAA were calculated [mean, standard deviation (SD)]. The independent t-test was used to compare mean variables between early and advanced group. To examine the associations between biomarkers, alignment and knee pain scores, β-coefficient was estimated after adjusting for age and the BMI using a multiple linear regression analysis. A P value <0.05 was considered to be statistically significant. All statistical analyses were performed using the SPSS15.0J software program (SPSS; Chicago, IL, USA).

Results

Baseline characteristics of the patients

A flow chart of the present study is shown in Fig. 1. While 202 patients who underwent the initial medical examination at our outpatient clinic between October 2009 and December 2011 were initially enrolled, 22 patients (10.9%) were excluded since they did not meet the study criteria, the remaining 180 patients were included in the data analysis. Among these 180 patients, as 20 patients (11.1%) were excluded due to invalid clinical evaluation scores or incomplete hematological data, so the remaining 160 patients (88.9%) were included in the analysis.

The patient characteristics are shown in Table I. The radiographic OA severities of the patients were 67 (41.9%) with a K/L grade of 2, 51 (31.9%) with a K/L grade of 3, and 42 (26.2%) with a K/L grade of 4. While no significant differences in the age of the patients
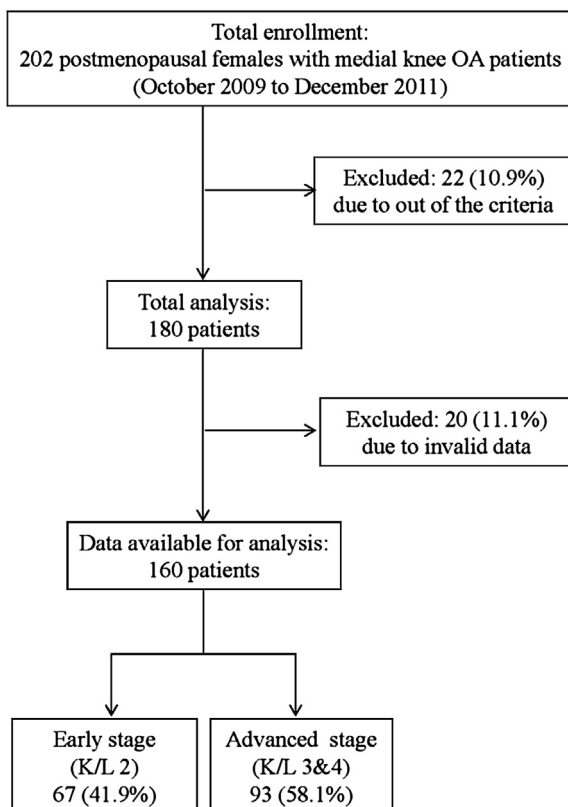
Table I
Baseline characteristic of the study subjects

	Total	Early	Advanced	P
n	160	67	93	
Age (years)	70.5 (7.6)	70.2 (6.7)	70.6 (8.2)	0.751
BMI (kg/m^2)	24.2 (3.5)	23.2 (3.1)	24.9 (3.5)	0.001*
Pain VAS score (Min: 0–Max: 100)	52.9 (24.7)	51.0 (26.8)	54.3 (23.1)	0.425
JKOM-pain score (Min: 0–Max: 32)	14.8 (6.1)	14.1 (5.8)	15.4 (6.4)	0.171
Serum IL-6 (pg/mL)	2.0 (1.0)	2.0 (1.0)	2.1 (0.9)	0.494
Serum hs-CRP (ng/mL)	686.1 (665.8)	629.7 (594.9)	726.8 (712.0)	0.350
AAA (degree)	180.5 (4.1)	182.9 (2.3)	178.8 (4.3)	<0.001*

Data are presented as the means and SD. Early: early-stage knee OA (K/L grade 2); Advanced: advanced-stage knee OA (K/L grade 3 or 4).
 * A P values <0.05 were considered to be statistically significant.

were observed between the early- (K/L grade 2) and advanced-stage (K/L grades 3 and 4) knee OA group, the BMI of the patients with an advanced-stage knee OA was significantly higher in comparison to that of those with an early-stage knee OA. No significant differences in the pain VAS score, the JKOM-pain score, serum IL-6 and hs-CRP levels were observed between the patients with early-stage and advanced-stage knee OA. On the other hand, the AAA of the patients with an advanced-stage knee OA was significantly smaller in comparison to that of the patients with an early-stage knee OA.

Factors associated with the pain severity in patients with knee OA

A multiple linear regression analysis indicated that the serum IL-6 levels and the AAA both associated with the pain severity, as evaluated by the pain VAS score in the patients when whole patients were included in the analysis (Table II). On the other hand, the serum levels of IL-6 were solely associated with the pain VAS score in the patients with early-stage knee OA, while the AAA was solely associated with the pain VAS score in the patients with advanced-stage knee OA.

Similarly, when the JKOM-pain score was used to evaluate the pain severity, the serum IL-6 levels and the AAA were both associated with the pain severity in the whole patients (Table III). In addition, the serum levels of IL-6 and the AAA solely associated with the JKOM-pain score in the patients with early- and advanced-stage knee OA respectively, thus confirming that there were differences in the pain-associated factors according to the stage of the disease.

Discussion

The present study has revealed, for the first time, that the factors associated with the pain severity in patients with knee OA varied with the radiographic disease severity. IL-6, which is related to synovitis, was mainly associated with the pain severity in early-stage knee OA, while the AAA was mainly associated with that in the advanced-stage knee OA.

Synovitis is seen from the early stage of knee OA[9,13,14,28,29]. The changes in cartilage matrix turnover detected by several potential molecular biomarkers were supposed to account directly or indirectly for synovitis and knee pain in patients with an early-stage knee OA[29]. More severe chronic synovitis was also seen in the advanced stage of knee OA than that in the early stage[13,30,31]. The severity of chronic synovitis was correlated with the pain severity and the level of disability in late-stage knee OA patients[32]. However, an increased degree of mononuclear cell infiltration and an overproduction of proinflammatory cytokines were more

Fig. 1. A flow chart of the present study.

Table II
Factors associating with the pain VAS score in patients with knee OA

Pain VAS score		β (95% CI) (crude)	P	β (95% CI) (adjusted)	P
Total	IL-6	5.87 (1.57–10.18)	<0.01	5.92 (1.48–10.35)	<0.01*
	hs-CRP	−0.002 (−0.008−0.004)	0.55	−0.002 (−0.008−0.005)	0.57
	AAA	−1.03 (−1.95 to −0.12)	0.03	−1.04 (−2.03 to −0.06)	0.04*
Early	IL-6	10.65 (4.16–17.15)	<0.01	10.77 (4.14–17.40)	<0.01*
	hs-CRP	0.000 (−0.011−0.012)	0.95	0.002 (−0.011−0.015)	0.74
	AAA	−0.004 (−2.683−2.68)	0.99	−0.03 (−2.74−2.69)	0.99
Advanced	IL-6	0.895 (−4.87−6.66)	0.76	0.64 (−5.44−6.72)	0.86
	hs-CRP	−0.001 (−0.009−0.006)	0.73	−0.01 (−0.009−0.006)	0.70
	AAA	−1.36 (−2.45 to −0.27)	0.02	−1.29 (−2.51 to −0.08)	0.04*

A multiple linear regression analysis. Adjusted; adjusted for age and BMI; Early: early-stage knee OA (K/L grade 2); Advanced: advanced-stage knee OA (K/L grade 3 or 4).
 * A P values <0.05 were considered to be statistically significant.

frequently observed in the early-stage of knee OA than in the late-stage knee OA[28,33]. These studies suggest that the proinflammatory cytokines may be mainly involved in the synovitis in the early-stage of knee OA, while synovitis is associated with the pain severity in early- to advanced-stage in knee OA, as observed in this study (Tables II and III). The associations between serum levels of IL-6 and the pain severity in the patients with early-stage knee OA, but not in those with advanced-stage knee OA, observed in the present study (Tables II and III), may be related to the results of these previous studies.

Although the serum hs-CRP, as well as IL-6, levels became high according to the progression of OA, in patients with early stage, the levels of IL-6, but not those of hs-CRP, significantly associated with the pain severity. The reason for the difference in this association is not clear. Circulating IL-6 is known to stimulate CRP production by the liver. CRP is a component of circulating serum β-globulin which is produced in the liver reacts to systemic and local tissue damage. IL-6 is a cytokine which is produced at a local site where cellular or tissue damage occurs. In addition, IL-6 is also produced by adipose tissue, which accompanies obesity[34]. As BMI of the patients with advanced-stage knee OA was significantly increased in comparison to that of those with early-stage knee OA in the present study (Table I), serum IL-6 levels may be affected by the IL-6 produced by adipose tissues, in addition to a local site in the knee joint. Therefore, further study using synovial fluid, which is one of the sources to observe the local joint event, is necessary to clarify this question.

The mechanical loading across the knee joint, which was estimated by the knee adduction moment (KAM), was speculated to be higher due to the deteriorating lower limb alignment, and also because of increasing body weight[7]. However, it had not been elucidated to what extent the mechanical loading across the knee joint contributes to the knee joint pain in patients with knee OA. There was a weak correlation between the pain severity and either the radiographic joint space narrowing or the alignment of the

lower limb in patients with early- to advanced-stage knee OA[3]. Consistent with this study, the lower limb alignment was weakly associated with the pain severity in all the patients, with early- and advanced-stage knee OA in the present study. Moreover, the association between the alignment and the pain severity became clear in the patients with advanced-stage knee OA, who show a mild to severe varus alignment of the lower limb, while the association was not significant in the patients with early-stage knee OA, who generally show a normal to less severe varus alignment of the lower limb (Tables I–III). In the patients with advanced-stage knee OA, an association was identified between pain severity and the KAM, while no association was observed in the patients with early-stage knee OA[35]. Among the early-stage with knee OA (K/L grade 2), the KAM significantly increased in patients with knee pain compared to those without pain[36]. Simultaneously, the alignment of lower limb in the patients with knee pain also significantly increased (more varus lower limb alignment) in comparison to that in those without knee pain[36]. Although, it is still remains unclear precisely how joint loading induces pain in patients with knee OA and such joint loading was not measured in the present study, the results of the present study suggest that the pain in the advanced-stage of knee OA is associated with the mechanical loading across the knee joint, which is associated with a deterioration of the lower limb alignment[35].

The current study was associated with some limitations. First, the present study is cross-sectional. The study deals with changes of the disease pathology according to the progression. Progression is time-related and ideally, the study should be designed longitudinally. In this context, the results obtained in this study may be understood as an indirect evidence. Secondly, the patients were divided into two subgroups for statistical analysis. The number of patients with advanced-stage OA was more by about 50% than that with early-stage OA. This may have produced the possibility of generating statistical bias comparing the two subgroups. Thirdly,

Table III
Factors associating with the JKOM-pain score in patients with knee OA

JKOM-pain score		β (95% CI) (crude)	P	β (95% CI) (adjusted)	P
Total	IL-6	1.75 (0.72–2.78)	<0.01	1.73 (0.67–2.79)	<0.01*
	hs-CRP	0.000 (−0.002−0.001)	0.68	0.000 (−0.002−0.001)	0.70
	AAA	−0.40 (−0.62 to −0.18)	<0.01	−0.40 (−0.63 to −0.16)	<0.01*
Early	IL-6	3.26 (2.00–4.51)	<0.01	3.19 (1.93–4.44)	<0.001*
	hs-CRP	−0.001 (−0.003−0.002)	0.65	−0.001 (−0.004−0.001)	0.24
	AAA	−0.28 (−0.80−0.23)	0.28	−0.27 (−0.79−0.25)	0.29
Advanced	IL-6	0.25 (−1.31−1.80)	0.76	0.34 (−1.30−1.97)	0.69
	hs-CRP	0.000 (−0.002−0.002)	0.82	0.000 (−0.002−0.002)	0.76
	AAA	−0.47 (−0.76 to −0.17)	<0.01	−0.49 (−0.82 to −0.16)	<0.01*

A multiple linear regression analysis. Adjusted; adjusted for age and BMI; Early; early-stage knee OA (K/L grade 2); Advanced, advanced-stage knee OA (K/L grade 3 or 4).
 * A P values <0.05 were considered to be statistically significant.

the serum IL-6 levels have been reported to show a circadian rhythm[37], the serum sample collection was not timed but collected in the morning and the patients had not fasted. While it was not examined whether the serum IL-6 levels show diurnal and activity-related variations, some biomarkers have been reported to have such variations[38–40]. Further studies are required to clarify whether there are time of day- and activity-related variations in the expression of IL-6 and hs-CRP[38]. Because we did not conduct detailed phenotyping of other joints, the contribution of other joints to the systemic levels of biomarkers cannot be addressed.

In conclusion, the presence of a higher serum levels of IL-6 is thus considered to be associated with pain in early-stage knee OA, while the varus alignment of the joint was found to be associated with pain in advanced-stage knee OA patients.

Contributions

YS, HK and MI conceived and designed the study. YS, HK, MT, YS, MS, YI collected and registered patients data. YS, MI, LL, HK, IF, RS, SH had the major role in analysis and interpretation of the data, and contributed to drafting the report. KK and HK also supervised the statistical analysis. All authors have read and approved the final manuscript.

Conflict of interests
All authors declare that they have no competing interests.

Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.joca.2013.05.014.

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