Osteoarthritis and Cartilage 19 (2011) 51–57

Osteoarthritis and Cartilage

Knee osteoarthritis, knee joint pain and aging in relation to increasing serum hyaluronan level in the Japanese population

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ARTICLE INFO

Article history:
Received 26 March 2010
Accepted 26 October 2010

Keywords:
Serum hyaluronan
Knee osteoarthritis
Knee pain
Biomarker
Japanese
Health checkups

SUMMARY

Objective: To investigate relationship between serum hyaluronan (HA) level and the presence and severity of radiographic knee osteoarthritis (OA) as well as degree of knee pain in Japanese population.
Design: A total of 616 volunteers participated in this study. Based on the Kellgren–Lawrence (K–L) grade, participants were radiographically classified into three groups: Normal (K–L grade 0 or 1), Moderate (grade 2) and Severe (grade 3 or 4). The degree of knee pain was quantified by visual analogue scale (VAS) and Knee injury and Osteoarthritis Outcome Score (KOOS) Pain. Serum HA levels were compared among the Normal, Moderate and Severe groups, and the relationship between serum HA level and the severity of knee OA was analyzed after age, sex and body mass index (BMI) were adjusted. In addition, the correlation between serum HA level and the degree of knee pain was analyzed in each group.
Results: Regarding relationship between serum HA level and the severity of radiographic knee OA, serum HA levels of the Moderate and Severe groups were significantly higher than in the Normal group ($P < 0.001$). Furthermore, serum HA level correlated with the severity of radiographic knee OA ($r = 0.289$, $P < 0.001$) after adjusting for age, sex and BMI. Serum HA level correlated with VAS of knee pain and/or KOOS Pain in the Normal and Moderate groups.
Conclusion: Serum HA level has the potential to be useful for the diagnosis of the presence and severity of knee OA.

Introduction

Knee Osteoarthritis (OA) is one of the most common knee joint diseases in the elderly, and is characterized by progressive cartilage degradation and concomitant bony hypertrophy. In clinical practice, diagnosis and assessment of knee OA are conventionally based on clinical history and radiological findings[1,2]. Patients' chief complaints are pain and stiffness of their knees, and radiological findings of knee OA include joint space narrowing, osteophyte formation, subchondral sclerosis and cysts[3]. However, radiological findings don't always reflect patients' knee symptoms. Recently, several alternative techniques have been used to assess knee OA, especially in its early stages. Magnetic resonance imaging (MRI) is a useful technique for assessing cartilage lesions of knee OA[4,5]. In addition, ultrasonography is also used to assess cartilage lesions[6].

To date, various biomarkers of knee OA have been studied to potentially aid in early diagnosis and to assess minor changes in patients' bone or cartilage that are predictive factors for further development of knee OA[7–11]. Cibere *et al.* reported that serum and urinary biomarkers of pre-radiographically defined OA[12]. They suggested that specific biomarker ratios combining cartilage degradation markers and synthesis markers were better able to differentiate OA stages compared with individual marker levels. Early diagnosis and prediction of progression are of particular importance from the standpoint of prevention and therapeutic strategy. However, although several biomarkers for knee OA have been investigated, there is no established marker for pre-radiographic knee OA.

Hyaluronan (HA) is a high molecular weight glycosaminoglycan, composed of alternating subunits of glucosamine and glucuronic acid. It has been reported to be widely distributed in much of extracellular matrix (ECM). HA is produced locally by cells of the ECM, and it plays a role in structural properties as well as in cell signaling[13]. HA and HA fragments were released into the systemic circulation by degeneration and turnover of the ECM[14]. HA is

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1063-4584/$ – see front matter
doi:10.1016/j.joca.2010.10.021

present in high concentrations in joint tissues including synovium, cartilage, and synovial fluid. Recently, increase in serum level of HA has been suggested as a result of synovial inflammation and cartilage degradation, and thus, the measurement of the HA level in serum may be useful in assessing knee OA activity as well as determining predictive factors[15,16].

In previous studies, it has been reported that serum HA level was associated with the presence of radiographic knee OA[17–20]. However, radiographic knee OA indicates the stable structural condition of this disease rather than the activity of the disease at the time. The objectives of this study were to investigate the relationship between serum HA level and the degree of knee pain as well as the presence and severity of radiographic knee OA.

Methods

Participants

The Iwaki Health Promotion Project is a community-based program to increase average life expectancy by performing general health checkups. This program began in 2005 and is being conducted over a 10-year-period. About 1000 people who are 20 years or older living in the Iwaki area of Hirosaki city located west of Aomori, Japan participate every year. Physicians, surgeons, orthopedists, gynecologists, urologists, psychiatrists, dermatologists and dentists from Hirosaki University are involved in this project to investigate diseases and disorders in various fields. The research of risk factors and prevention for knee OA is one part of this project.

A total of 886 volunteers (20–86 years old, 325 males and 561 females) participated in the Iwaki Health Promotion Project in 2008. Exclusion criteria for our study were history of rheumatoid arthritis, hepatic disease, renal disease or malignant disease, total knee arthroplasty, total hip arthroplasty and femoral head replacement. In addition, knee OA patients under treatment and those taking oral nonsteroidal anti-inflammatory drugs (NSAIDs) were also excluded. Finally, 616 participants (242 males and 374 females) were included in this study. The mean (±SD) ages in male and female participants were 57.0 ± 12.4 (range: 27–85) and 58.5 ± 10.4 (21–80) years, and there were no differences between male and female ($P = 0.139$) (Table I). Approval for the study was obtained from the Ethics Committee of Hirosaki University School of Medicine and all subjects gave written informed consent before participating.

All participants answered questionnaires about their medical history, lifestyle, smoking, drinking, and fitness habits, occupational history, family history, health-related quality of life, various disease-specific information including knee symptoms and intake of supplements for the knee. Anthropometric measurement included height, weight, body mass index (BMI), body fat percentage, density of bone, waist-hip ratio, bilateral grip strength, functional reach test[21,22], and timed up and go (TUG) test[23,24]. TUG test was performed to evaluate ability to rise from a seated position, walk 3 m, turn, and walk back returning to a seated position.

Blood and urine samples were taken in all participants before breakfast in the early morning for biochemical examinations. The serum HA level was determined using the Hyaluronan Assay Kit (Seikagaku Corporation, Tokyo, Japan). In clinical examination by well-experienced orthopedists, information about the knee, hip, elbow, neck and low back including range of motion was taken. Plain radiographs of knee, hip, hand, cervical spine and lumber spine were taken for 817 out of 886 participants.

Knee symptoms

To evaluate degree of knee pain, visual analogue scale (VAS) in both knees and Knee Injury and Osteoarthritis Outcome Score (KOOS) subscale Pain were used. In VAS evaluation, the degree of knee pain for the last week was quantified on a scale of 0–100 mm, and VAS score of asymptomatic knees were evaluated for 0 mm. In analysis, the mean values of VAS in both knees were used.

KOOS is a 42-item knee-specific self-administered instrument with five subscales: Pain, Symptom, Activities of Daily Living (ADL), Sport and Recreation (Sport/Rec) and Knee-related Quality of Life (QOL). All items were scored from 0 to 4 and summed. Raw scores were then transformed to a 0–100 scale where 100 represents the best result. A separate score was calculated for each of the five subscales[25]. KOOS Pain was calculated by 9 out of the 42 items. However, there is no Japanese version of KOOS at the present. Therefore, two orthopedists independently of each other translated

Table I
Age, BMI, degree of knee pain, serum HA level and K–L grade of radiographic knee OA in study participants

Age, BMI, degree of knee pain and serum HA level				
		Male ($n = 242$)	Female ($n = 374$)	*P*-value
Age (year)		57.0 ± 12.4	58.5 ± 10.4	0.139
BMI (kg/m^2)		23.4 ± 2.7	22.8 ± 3.2	<0.001*
VAS of knee pain (mm)		9.0 ± 15.4	12.3 ± 19.9	0.063
KOOS Pain (point)		93.9 ± 12.0	91.1 ± 13.5	<0.001*
Serum HA level (ng/ml)		61.9 ± 35.9	65.0 ± 38.0	0.329
K–L grade and the prevalence rate of radiographic knee OA				
		Male ($n = 242$)	Female ($n = 374$)	*P*-value
K–L grading	Grade 0	90	62	
	Grade 1	114	173	
	Grade 2	34	104	
	Grade 3	3	32	
	Grade 4	1	3	
Severity	Normal group	204 (84.3%)	235 (62.8%)	
	Moderate group	34 (14.0%)	104 (27.8%)	
	Severe group	4 (1.7%)	35 (9.4%)	
Laterality	Unilateral group	11 (4.5%)	47 (12.6%)	
	Bilateral group	27 (11.2%)	92 (24.6%)	
	Prevalence rate	38 (15.7%)	139 (37.2%)	$P < 0.001^{\#}$

The values of Age, BMI, VAS of knee pain, KOOS Pain and serum HA level are the mean ± S.D. *P*-values below 0.05* indicate a significant level of difference between male and female, using Mann–Whitney *U* test. By the severity of the participants' worse knee, they were classified into three groups: Normal (K–L grade of 0 or 1), Moderate (K–L grade of 2) and Severe (K–L grade of 3 or 4). By the laterality of radiographic knee OA, they were classified into three groups: Normal, Unilateral and Bilateral. The presence of radiographic knee OA was defined as a K–L grade of 2, 3 and 4. *P*-values below 0.05# indicate a significant level of association between the prevalence rate of radiographic knee OA and sex, using χ^2 test.

Table II
Numbers of knees, hips and hands with radiographic OA, and correlation with serum HA level

Numbers of joints with radiographic OA					
Knee OA		Hip OA		Hand OA	
Normal	439	Normal	512	0–2 joints	568
Unilateral	58	Unilateral	65	3–6 joints	33
Bilateral	119	Bilateral	39	7–0 joints	15

Correlation between serum HA level and numbers of joints with OA					
Unadjusted			Age, Sex and BMI adjusted		
	r	*P*-value		*r*	*P*-value
Knee OA	0.395	<0.001*	Knee OA	0.220	<0.001*
Hip OA	0.108	0.007*	Hip OA	0.065	0.109
Hand OA	0.262	<0.001*	Hand OA	0.213	<0.001*

r = correlation coefficient. The numbers of knees and hips with OA are represented as 0–2 joints. Scores of Hand OA are represented as 0 (0–2 hand joints affected), 1 (3–6) and 2 (7-). The relationship between serum HA level and the numbers of knee and hip OA joints, scores of hand OA were analyzed by Spearman rank correlation coefficient (unadjusted) and partial correlation analysis (age, sex and BMI adjusted). *P*-values below 0.05* indicate a significant level.

Table III
Comparison of serum HA level among age-groups and between Normal and knee OA participants in each age-group

	Normal	(*n*)	Knee OA	(*n*)
20 s	32.3 ± 9.0	(5)	–	(0)
30 s	35.1 ± 8.4	(24)	27.1	(1)
40 s	38.1 ± 8.5	(111)	46.1 ± 10.5*	(5)
50 s	52.9 ± 23.5†‡	(156)	61.5 ± 27.0	(37)
60 s	68.5 ± 29.6*†‡§	(96)	79.4 ± 41.0	(76)
70 s	79.2 ± 31.0*†‡§	(44)	114.8 ± 49.9#††‡‡*	(52)
80 s	127.0 ± 53.8*†‡§‖¶	(3)	138.6 ± 53.9#††‡‡	(6)

Values of serum HA level are the mean ± S.D (ng/ml). Comparisons of serum HA level among age-groups (20 s, 30 s, 40 s, 50 s, 60 s, 70 s and 80 s) and between Normal and knee OA participants in each age-group were analyzed using two-way ANOVA. Tukey's test was performed among age-groups and Mann–Whitney *U* test was performed between Normal and knee OA participants for *post hoc* analysis.
P-value below 0.05 $^{*-\ddagger\ddagger}$, * indicates significance level.
* $P < 0.05$ between Normal and Knee OA.
* $P < 0.05$ vs Normal in 20 s.
† $P < 0.05$ vs Normal in 30 s.
‡ $P < 0.05$ vs Normal in 40 s.
§ $P < 0.05$ vs Normal in 50 s.
$^{\|}$ $P < 0.05$ vs Normal in 60 s.
¶ $P < 0.05$ vs Normal in 70 s.
$^{\#}$ $P < 0.05$ vs Knee OA in 40 s.
†† $P < 0.05$ vs Knee OA in 50 s.
‡‡ $P < 0.05$ vs Knee OA in 60 s.

the English version of the KOOS questionnaire into Japanese. Both orthopedists had a medical background in knee disease and both were native Japanese speakers. Secondly, two bilingual persons who were blinded to the original English version re-translated independently of each other this Japanese version into English[26]. Finally, all translators had a consensus meeting to consolidate the final Japanese translation of the KOOS questionnaire, which was used.

Radiographic knee, hip and hand OA

On the day of general health checkups, weight-bearing and anterior–posterior radiographs of both knees and hips and dorsal-volar hands of participants were taken. Scores were given to each knee and hip radiograph based on the Kellgren–Lawrence (K–L) grade of either 0, 1, 2, 3 or 4[3]. The presence of radiographic knee and hip OA were defined as a K–L grade of 2, 3 and 4 and participants were classified into Normal (0 affected), Unilateral (1 affected) and Bilateral (2 affected) knee and/or hip OA by the laterality, respectively. For hand OA, a sum score was derived based on the number of joint sites with K–L grades of 2, 3 and 4. The sum score of the hand ranged from 0 to 20, consisting of the distal interphalangeal joint (DIP) 2–5, proximal interphalangeal joint (PIP) 2–5, interphalangeal joint 1, and carpometacarpal joint 1. The hand OA score (0–2) represents participants with, respectively, 0–2, 3–6, and 7-hand joints affected[27]. By the severity of radiographic knee OA in the participants' worse knee, they were classified into three groups: Normal (K–L grade of 0 or 1), Moderate (K–L grade of 2) and Severe (K–L grade of 3 or 4). Although lateral radiographs were taken of the cervical and lumber spines, it was hard to define spine OA because the lumber facet joint OA could not be evaluated, and C5/6 and C6/7 of some participants were invisible. Only radiographs of knees, hips and hands were used in this study.

Fitness, smoking and drinking habits

Regarding fitness habits, participants who exercise once or more a week were included in the fitness habit group. Regarding smoking and drinking, only participants who had habits at the present time were included in the habit group, and those who had habits in the past were included in the non-habit group.

Statistical analysis

Data input and calculation were performed with the SPSS ver.12.0J (SPSS Inc., Chicago, IL, USA). The comparison of age, BMI, VAS of knee pain, KOOS Pain and serum HA level between male and female were performed using Mann–Whitney *U* test. The prevalence rate of radiographic knee OA between male and female was analyzed using χ^2 test. The relationship between serum HA level and the number of knee and hip OA joints, and score of hand OA were analyzed by Spearman rank correlation coefficient, and partial correlation analysis to adjust age, sex and BMI. One-way analysis of variance (ANOVA) was used to analyze age, BMI, VAS of knee pain, KOOS Pain and serum HA level among Normal, Moderate and Severe groups, and Tukey's test for *post hoc* analysis was performed. Comparisons of serum HA level among age-groups (20 s, 30 s, 40 s, 50 s, 60 s, 70 s and 80 s) and between Normal and knee OA participants in each age group were analyzed using two-way ANOVA. Tukey's test was performed to compare serum HA level among age-groups and Mann–Whitney *U* test was performed between Normal and knee OA participants for *post hoc* analysis. The relationship between serum HA level and the severity of radiographic knee OA was analyzed by Spearman rank correlation coefficient, and partial correlation analysis to adjust age, sex and BMI. In addition, to evaluate the relationship between serum HA level and the degree of knee pain, the relationships between serum HA level and VAS of knee pain and/or KOOS Pain were analyzed by Spearman rank correlation coefficient. Furthermore, multiple regression analysis was performed with serum HA level as the independent variable, and VAS of knee pain, age, sex, BMI, laterality of radiographic knee OA, intake of supplements for knee, fitness, smoking and drinking habits as the dependent variables in the Normal, Moderate and Severe groups, respectively. In addition, KOOS Pain was substituted for VAS of knee pain as the independent variable in the same way. In all analysis, *P*-values < 0.05 were considered significant.

Results

Regarding the severity of radiographic knee OA, 204 (84.3%) out of 242 males were classified as Normal, 34 (14.0%) as Moderate and 4 (1.7%) as Severe, and 235 (62.8%) out of 374 females were classified as Normal, 104 (27.8%) as Moderate and 35 (9.4%) as Severe (Table I). The prevalence rates of radiographic knee OA were 15.7% (Unilateral: 4.5%, Bilateral: 11.2%) in males and 37.2% (Unilateral: 12.6%, Bilateral: 24.6%) in females. The prevalence rate of radiographic knee OA was significantly higher in females than in males ($P < 0.001$) (Table I). Characteristically, in both males and females, the Moderate (both male and female: $P < 0.001$) and Severe (male: $P = 0.017$, female: $P < 0.001$) groups were significantly older than the Normal groups. There were no significant differences in BMI among the three groups in males. However, that of the Moderate ($P = 0.022$) and Severe ($P < 0.001$) groups in females were significantly higher than the Normal group (Table IV).

Regarding the degree of knee pain, the mean values of VAS of knee pain in males and females were 9.0 ± 15.4 and 12.3 ± 19.9, respectively, and there was no significant difference between males and females ($P = 0.063$) (Table I). Regarding the relationship between VAS of knee pain and the severity of radiographic knee OA, the mean values of VAS of knee pain of the Moderate (male: $P = 0.049$, female: $P = 0.002$) and Severe (both male and female: $P < 0.001$) groups were significantly higher than the Normal group (Table IV). The mean values of KOOS Pain in males and females were 93.9 ± 12.0 and 91.1 ± 13.5, and that of females was significantly

Table IV
Relationship between serum HA level and severity of radiographic knee OA

		Normal	Moderate	*P*-value	Severe	*P*-value
Male	Age (year)	55.2 ± 11.9	66.4 ± 10.6	<0.001*	71.5 ± 7.9	0.017*
	BMI (kg/m^2)	23.4 ± 2.7	23.3 ± 2.4	0.967	23.8 ± 2.7	0.951
	VAS of knee pain (mm)	7.5 ± 13.4	13.9 ± 17.5	0.049*	45.8 ± 37.6	<0.001*
	KOOS Pain	95.6 ± 8.6	87.3 ± 16.9	<0.001*	62.5 ± 37.9	<0.001*
	Serum HA level (ng/ml)	55.2 ± 27.6	90.2 ± 38.9	<0.001*	166.1 ± 94.9	<0.001*
Female	Age (year)	54.8 ± 10.0	63.7 ± 7.9	<0.001*	68.2 ± 6.3	<0.001*
	BMI (kg/m^2)	22.2 ± 2.7	23.2 ± 3.5	0.022*	25.1 ± 4.0	<0.001*
	VAS of knee pain (mm)	8.1 ± 15.2	15.8 ± 22.7	0.002*	30.2 ± 26.4	<0.001*
	KOOS Pain	95.1 ± 9.0	87.4 ± 15.3	<0.001*	75.0 ± 17.5	<0.001*
	Serum HA level (ng/ml)	53.9 ± 27.3	72.4 ± 36.4	<0.001*	117.4 ± 53.6	<0.001*

Correlation between serum HA level and severity of radiographic knee OA

Unadjusted			Age, Sex and BMI adjusted		
	r	*P*-value		*r*	*P*-value
Severity of Knee OA	0.410	<0.001#	Severity of Knee OA	0.289	<0.001#

The values of Age, BMI, VAS of knee pain, KOOS Pain and serum HA level are the mean ± S.D. *P*-value below 0.05* indicates significant difference from Normal. *r* = correlation coefficient. *P*-values below 0.05# indicate a significant level by Spearman rank correlation coefficient (unadjusted) and partial correlation analysis (Age, Sex and BMI adjusted).

lower than in males ($P < 0.001$) (Table I). In relationship between KOOS Pain and the severity of radiographic knee OA, the mean values of KOOS Pain of the Moderate and Severe groups in both males and females were significantly lower than in the Normal groups ($P < 0.001$, respectively) (Table IV).

Serum HA level correlated with aging ($r = 0.676$, $r < 0.001$) and the number of knee OA ($r = 0.395$, $r < 0.001$) and hip OA ($r = 0.108$, $P = 0.007$) joints and/or the scores of hand OA ($r = 0.262$, $r < 0.001$). In addition, it correlated with the number of knee OA ($r = 0.220$, $P < 0.001$) joints and the score of hand OA ($r = 0.213$, $r < 0.001$) after age, sex and BMI were adjusted (Table II). The mean values (±SD) of serum HA levels in males and females were 61.9 ± 35.9 and 65.0 ± 38.0 ng/ml respectively, and there was no significant difference between males and females ($P = 0.329$) (Table I). In addition, the mean values in males and females were 55.2 ± 27.6 and 53.9 ± 27.3 ng/ml in Normal, 90.2 ± 38.9 and 72.4 ± 36.4 ng/ml in Moderate, 166.1 ± 94.9 and 117.4 ± 53.6 ng/ml in Severe group, respectively. The mean values of serum HA levels of the Moderate and Severe groups were significantly higher than the Normal groups in both males and females ($P < 0.001$, respectively) (Table IV). Regarding comparison of serum HA levels in each age group, serum HA levels gradually increased with aging. Furthermore, serum HA levels of the knee OA group in the 40 s ($P = 0.048$) and 70 s ($P < 0.001$) were significantly higher than in the Normal group (Table III).

Regarding the relationship between serum HA level and the severity of radiographic knee OA, serum HA level correlated with the severity of radiographic knee OA ($r = 0.410$, $r < 0.001$), and it also correlated with that ($r = 0.289$, $P < 0.001$) after age, sex and BMI were adjusted (Table IV). Regarding the relationship between serum HA level and the degree of knee pain in each group by the severity of radiographic knee OA, significant correlation between serum HA level and VAS of knee pain was seen in the Total ($r = 0.199$, $P < 0.001$) and the Moderate ($r = 0.177$, $P = 0.037$) groups, and significant correlation between serum HA level and KOOS Pain was seen in the Total ($r = -0.255$, $P < 0.001$) and the Moderate ($r = -0.252$, $P = 0.003$) groups (Table V). After age, sex, BMI, laterality of radiographic knee OA, intake of supplements for the knee, fitness, smoking and drinking habits were adjusted, significant correlation between serum HA level and VAS of knee pain was seen in the Normal ($P = 0.002$) and Moderate ($P = 0.003$) groups, in the same way, significant correlation between serum HA level and KOOS Pain was seen in the Normal ($P < 0.001$) and Moderate ($P < 0.001$) groups (Table V). As for the remainder, positive correlation between serum HA level and age was seen in the Normal and Moderate groups ($P < 0.001$, respectively). However, there were no correlations between serum HA level and VAS of knee pain ($P = 0.460$), KOOS Pain ($P = 0.077$) or age ($P = 0.051$ with VAS of knee pain, $P = 0.075$ with KOOS Pain) in the Severe group in the same analysis (Table V).

Discussion

This study showed that serum HA level was positively associated with the occurrence of radiographic knee OA, and with the degree of knee pain in the Normal and Moderate knee OA groups. In several previous studies, relationships between serum HA level and radiographic knee OA have been investigated by various methods. Elliott *et al.* reported that serum HA level was positively associated with the presence and severity of radiographic knee OA in their large-scale study[19]. George and Pavelka reported that serum HA level had a predictive value for further development of knee OA[17,18]. On the other hand, Turan *et al.* reported that serum HA level of patients in a knee OA group was significantly higher than that of the normal healthy control group. However, they reported that there was no significant difference in serum HA levels between groups with K–L grade 2 and K–L grade 3–4[15]. In this study, significant differences of serum HA level were seen not only among Normal, Unilateral and Bilateral groups but also among Normal, Moderate and Severe groups by radiographic knee OA after age, sex and BMI were adjusted. Our study suggested that the presence and severity of radiographic knee OA caused an increase in serum HA level as in the large-scale study by Elliott *et al.* Regarding correlation between serum HA level and the number of OA joints, serum HA level was correlated with the number of knee and hip OA joints and the score of hand OA, respectively. Kraus *et al.* reported that serum HA level correlated with the joints affected by osteophytes in hands, hips, knees and lumber spine[28]. In this study, by Spearman rank correlation coefficient, serum HA level was more closely-linked to knee OA ($r = 0.395$) than hip ($r = 0.108$) or hand OA ($r = 0.262$). These results suggested that knee OA joints were more closely associated with serum HA level increase.

In relationship between serum HA level and the degree of knee pain in each group by the severity of radiographic knee OA, serum HA level was positively correlated with VAS of knee pain and KOOS Pain in Normal and Moderate groups in this study. Because the degree of knee pain seems to reflect synovial inflammation[29] and cartilage degeneration at the time[30], these positive correlations suggest that measurement of serum HA level is useful as a biomarker in moderate stage of knee OA. In addition, the knee pain can be objectively evaluated by measuring serum HA level in normal or moderate OA patients. On the other hand, there was no correlation between serum HA level and VAS of knee pain and KOOS Pain in the Severe group. In those results, the baseline of serum HA level in the Severe group was very high and correlation was not observed. High level of serum HA may reflect not only a high degree of knee pain but also the severity of radiographic knee OA. Therefore, it is suggested that the serum HA level is not suitable as a pain biomarker in severe radiographic knee OA patients. Turan *et al.* reported that there was no significant correlation between serum HA level of a knee OA group and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Pain score[15]. As for the reason, their analysis was a simple correlation coefficient between serum HA level and WOMAC Pain score

Table V
Relationship between serum HA level and the degree of knee pain (VAS, KOOS Pain)

Correlation between serum HA level and Age, VAS of knee pain and KOOS Pain						
	Age		VAS of knee pain		KOOS Pain	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Serum HA level in Normal	0.642	<0.001*	0.069	0.149	−0.087	0.067
Serum HA level in Moderate	0.550	<0.001*	0.177	0.037*	−0.252	0.003*
Serum HA level in Severe	0.218	0.183	0.086	0.604	−0.293	0.070
Serum HA level in Total	0.676	<0.001*	0.199	<0.001*	−0.255	<0.001*

Relationship between serum HA level and Age, VAS of knee pain and KOOS Pain in Normal, Moderate and Severe Knee OA groups (some factors adjusted)						
		B	95%CI			*P*-value
Serum HA level in Normal	*VAS*	0.236	0.085	–	0.387	0.002#
	Age	1.369	1.162	–	1.577	<0.001#
	KOOS Pain	−0.264	-0.520	–	−0.007	0.044#
	Age	1.350	1.142	–	1.559	<0.001#
Serum HA level in Moderate	*VAS*	0.391	0.138	–	0.645	0.003#
	Age	2.299	1.662	–	2.935	<0.001#
	KOOS Pain	−0.625	−0.965	–	−0.286	<0.001#
	Age	2.280	1.653	–	2.907	<0.001#
Serum HA level in Severe	*VAS*	0.264	−0.455	–	0.982	0.460
	Age	3.148	−0.009	–	6.304	0.051
	KOOS Pain	−0.945	−1.998	–	0.107	0.077
	Age	2.738	−0.290	–	5.766	0.075

r = correlation coefficient. The relationships between serum HA level and Age, VAS of knee pain and/or KOOS Pain in Normal, Moderate and Severe knee OA groups and Total participants were analyzed by Spearman rank correlation coefficient. *P*-values below 0.05* indicate a significant level. Multiple regression analysis was performed with serum HA level as the independent variable, and VAS of knee pain or KOOS Pain, age, sex, BMI, laterality of radiographic knee OA, intake of supplements for knee, fitness, smoking and drinking habits as the dependent variables in the normal, moderate and severe groups, respectively. *P*-values below 0.05# indicate a significant level of correlation with serum HA level. B: regression coefficients, 95%CI: 95% confidence intervals.

without adjustment for age, BMI, habits or supplements for the knee OA, in contrast to this study. Furthermore, VAS of knee pain and KOOS Pain were used to evaluate degree of knee pain in this study.

To provide more accurate evaluation of degree of knee pain and serum HA level, many participants were excluded from this study. Firstly, to evaluate the degree of knee pain more accurately, patients currently under knee OA treatment and participants taking oral NSAIDs were excluded. Although several previous studies of knee OA biomarkers included patients under treatment[31,32], we cannot completely assess details of treatments because they include oral medicine, external medicine and joint injections. Furthermore, patients with diseases of elevated serum HA level were also excluded at the beginning of this study, so that factors which influence serum HA level could be evaluated more accurately. It has been reported that HA enters the circulation as a result of synovial inflammation and cartilage degeneration, and serum HA level can be increased in RA patients[33,34]. In addition, because HA is widely distributed in the whole body, serum HA levels have been shown to be increased in patients with hepatic disease[35,36], renal disease[37] and malignant disease[38,39].

Regarding the relationship between serum HA level and aging, this study clearly showed that serum HA levels increased with age. Serum HA levels of participants in their 50 s, 60 s, 70 s and 80 s were significantly higher than in the younger groups. This result was similar to the previous studies showing that levels in those 50 years or older were significantly higher than in younger subjects[40]. In addition, after adjustment for several factors including the presence of radiographic knee OA, the serum HA levels were positively correlated with aging in this study. Therefore, when serum HA level is evaluated, effects produced by aging always have to be adjusted. Furthermore, important consideration in the evaluation of the age related increase in serum HA level is that it may be due to OA in other joints and spine. In previous studies, it has been shown that serum HA level was influenced by various factors other than the diseases described above. For example, activity level, eatings and intake of supplements for the knee were reported to increase serum HA level[31,41–43]. In this study, all blood samples of participants were taken before breakfast in the early morning, and the influence of activity and intake of supplements for the knee was statistically adjusted.

There were several limitations in this study. One was that it was performed in a limited region, which may not be representative of Japan as a whole. Because this study was to investigate the data of the general population, there was little data of knee OA patients, especially K–L grade 3 or 4[44,45]. This may have caused the lack of correlation between serum HA level and degree of knee pain in the Severe group. The KOOS instrument translated to Japanese that we used has not yet been validated in detail, and psychometric properties were invalid[46,47]. However, evaluation by KOOS Pain played much the same role as VAS of knee pain in this study. Inflammatory indexes, such as erythrocyte sedimentation rate (ESR) or high-sensitive C-reactive protein, were not measured in this study. Although VAS of knee pain and KOOS Pain were taken as degree of synovial inflammation and/or cartilage degeneration, there was no objective evidence. Finally, analyses in this study did not include evaluation of the presence of spine OA joints, which would likely increase serum biomarker levels[27,48].

Despite these limitations, this general population-based study clearly showed strong associations between serum HA level and the presence and severity of radiographic knee OA. Furthermore, this is the first study, as far as we know, to show that serum HA level in normal or moderate knee OA patients were positively correlated with the degree of knee pain. Based on this result, determination of serum HA level can be considered useful as an assessment option for OA knee pain, especially for evaluation of moderate knee OA.

Conclusions

Serum HA level was strongly associated with the presence and severity of radiographic knee OA. Additionally, serum HA level correlated with the degree of knee pain in radiographically normal

and moderate knee OA patients. It is suggested that serum HA levels have the potential to be useful for the diagnosis of the presence and severity of knee OA.

Author contributions

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Conflict of interest

There are no competing interests to declare.

Acknowledgements

We thank Eiki Tsushima, PhD for statistical analysis assistance, and Keiichiro Maniwa, MD for technical assistance. This study was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No.18200044), Japanese Society for the Promotion of Science (No.21500676) and JOA-Subsidized Science Project Research from the Japanese Orthopaedic Association.

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