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Associations between serum S100A8/S100A9 and knee symptoms, joint structures and cartilage enzymes in patients with knee osteoarthritis

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SUMMARY

Objective: Animal studies suggest that S100A8/S100A9 may be involved in the pathogenesis of osteoarthritis (OA); however, there has been no clinical study examining the associations between serum S100A8/S100A9 and knee symptoms, joint structures and cartilage degradation enzymes in knee OA patients so far. Therefore, this study was designed to investigate the cross-sectional associations between serum levels of S100A8/S100A9 and the outcomes in patients with knee OA.

Design: A total of 141 subjects with clinical knee OA were included. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score was used to assess joint symptoms. Magnetic resonance imaging (MRI) was used to measure knee structural abnormalities including cartilage defects. Knee radiography was used to assess joint space narrowing (JSN), osteophytes and the radiographic severity of OA. Enzyme-linked immunosorbent assay (ELISA) was used to measure the serum levels of S100A8/S100A9, matrix metalloproteinase (MMP)-3, MMP10 and MMP13.

Results: In multivariable analyses, serum S100A8/S100A9 were positively associated with total WOMAC score (β: 0.111 per 10 ng/ml, $P = 0.021$), WOMAC weight-bearing pain (β: 0.015 per 10 ng/ml, $P = 0.043$) and WOMAC physical dysfunction (β: 0.091 per 10 ng/ml, $P = 0.010$), and had positive associations with total cartilage defects and cartilage defects at lateral femoral, lateral tibial and medial femoral sites (ORs: 1.006–1.008 per 10 ng/ml, all $P < 0.05$) and serum levels of MMP3 (β: 0.002 per 10 ng/ml, $P = 0.032$) in patients with clinical knee OA.

Conclusions: Serum levels of S100A8/S100A9 were positively associated with increased knee symptoms, cartilage defects and serum cartilage degradation enzymes in patients with knee OA, suggesting that S100A8/S100A9 may have a role to play in knee OA. Future longitudinal studies are required to confirm these findings.

Introduction

Osteoarthritis (OA) is the most common form of arthritis characterized by cartilage damage, osteophyte formation, and other joint structural changes, and is most common in the knees[1]. It is the leading cause of joint pain and physical disability, which causes a large socioeconomic burden[2,3]. Although the pathogenesis of OA is unclear, low grade chronic inflammation is thought to play a central role in OA[2,4]. Age, sex and body mass index (BMI) are well-known risk factors for OA[5].

Alarmins S100A8 and S100A9 are proteins that belong to the family of damage-associated molecular patterns (DAMPs) and are produced mainly by monocytes, activated macrophages and neutrophils[6]. The preferred form for human S100A8 and S100A9 is the S100A8/S100A9 heterodimer, which exerts its effector functions mostly via binding to receptors like Toll-like receptor 4 (TLR4) and Receptor of Advanced Glycation End Products (RAGE)[7,8].

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High levels of S100A8/S100A9 have been found in the serum, synovial fluid and synovium of patients with OA[9,10]. In collagenase-induced osteoarthritis (CIOA) mice, S100A8/S100A9 were upregulated in serum and synovium compared to controls, and the synovial activation, cartilage destruction and osteophyte size were reduced in CIOA S100A9$^{-/-}$ mice (double knockout S100A8 and S100A9 functionally) compared to CIOA wild type mice[9,11]. Besides, treating CIOA mice with a S100A9-blocking compound paquinimod significantly reduced synovial activation, osteophyte formation and cartilage damage of the OA knee[10]. Although S100A8/S100A9 may be involve in the pathogenesis of OA, there is no clinical study examining the associations between serum S100A8/S100A9 and knee symptoms, joint structures and cartilage degradation enzymes in knee OA patients. The aim of this study, therefore, was to investigate the cross-sectional associations of serum levels of S100A8/S100A9 with knee symptoms, joint structural changes and serum cartilage degradation enzymes in patients with symptomatic knee OA.

Methods

Subjects

This study was part of the Anhui Osteoarthritis (AHOA) Study, a clinical study of 205 patients that aimed to identify the environmental and biochemical factors associated with the progression of knee OA. Patients with clinical knee OA, diagnosed using American College of Rheumatology criteria[12], were consecutively recruited from the Department of Rheumatology and Immunology in the First Affiliated Hospital of Anhui Medical University, from January 2012 to November 2013. We excluded institutionalized patients, patients with rheumatoid arthritis or other inflammatory diseases (such as systemic lupus erythematosus and spondylolysis which were confirmed by rheumatologists), patients with severe knee OA who were planning to have knee arthroplasty in 2 years (this study was ongoing with 2 years of follow-up), and patients with contraindications to magnetic resonance imaging (MRI) (including metal sutures, presence of shrapnel, iron filings in the eye, and claustrophobia). Sixty-four patients were excluded from the study because of incomplete data, leaving 141 patients in the final analysis. The study was approved by the First Affiliated Hospital of Anhui Medical University Ethics Committee (Hefei, Anhui, China), and written informed consents were obtained from all participants.

Anthropometrics

Weight was measured to the nearest 0.1 kg (with shoes, socks and bulky clothing removed) by using a single pair of electronic scales that were calibrated using a known weight at the beginning. Height was measured to the nearest 0.1 cm (with shoes, socks and headgear removed) by using a stadiometer. BMI was calculated [weight (kg)/height (m)^2].

Joint symptom assessments

Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was acquired by questionnaires, and the subscores of knee joint pain (5 items), stiffness (2 items), and physical dysfunction (17 items) were self-reported. Knee joint pain was divided into weight-bearing pain (walking on flat surface, going up/down stairs, and when standing upright) and non-weight-bearing pain (at night while in bed and when sitting/lying). The 10-point scale (0–9) for each items were used in this study with higher scores indicating greater complications.

Serum S100A8/S100A9 and cartilage degradation enzymes measurements

Fasting blood samples were collected from patients in the morning. Serum was separated, aliquoted into 1.5 mL eppendorf tubes, and stored at −80°C until analysis. Serum levels of S100A8/S100A9 and matrix metalloproteinase (MMP)-3, 10, 13 were measured by using enzyme-linked immunosorbent assay (ELISA) (R&D Systems, USA for S100A8/S100A9, and elabscience, China for others) kits according to the manufacturer's instructions.

Knee radiographic assessment

All patients underwent knee radiographic examination. The 15-degree flexion, standing, anteroposterior view image was taken in the symptomatic knees (the severer one if both knees were affected; the right one if both knees were equally painful). Kellgren & Lawrence (K–L) grading system (grades 0–4) was used to assess the radiographic severity of OA[13]. Radiographic OA (ROA) was defined as K–L grade of $\geq$2. Joint space narrowing (JSN) and osteophytes were also assessed on a scale of 0–3 using the Osteoarthritis Research Society International atlas[14].

MRI assessment

MRI of the selected knee was performed with a 3.0-T whole-body MRI unit (Signa HDxT 3.0T; GE Healthcare, Little Chalfont, UK), using a commercial transmit/receive extremity coil. The following sequence and parameters were used: (1) A T1-weighted fat saturation three-dimensional spoiled gradient-recalled (SPGR) acquisition in the steady state with flip angle 30°; repetition time 31 ms; echo time 6.71 ms; field of view 16 cm; 60 partitions; 512 × 512-pixel matrix; acquisition time 11 min and 56 ms, 1 acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 × 0.31 (512 × 512 pixels). (2) A T2-weighted fat saturation two-dimensional fast spin echo, flip angle 90°, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 256 × 256-pixel matrix. Sagittal images were obtained at a slice thickness of 4 mm with an interslice gap of 1.0 mm. Images were checked for image noise and structural abnormalities interfering with segmentation. All images were grouped together and read in randomized order, with the reader blinded to subject information and status.

Regional subdivision of the articular surfaces: femur and tibia are divided into medial and lateral sites, with the trochlear groove of the femur considered as a part of the medial site. The femoral and tibial surfaces are further subdivided into anterior (A), central (C) and posterior (P) subregions. Region A of the femur corresponds to the patellofemoral articulation; region C the weight bearing surface, and region P the posterior convexity that articulates only in extreme flexion[15].

Cartilage defects (0–4 scale) were assessed at the medial femoral, lateral femoral, medial tibial, lateral tibial, and patellar sites using T2-weighted images as follows[16]: grade 0, normal cartilage; grade 1, focal blistering and intracartilaginous increased-signal intensity area with intact surface; grade 2, irregularities on the surface or bottom and loss of thickness less than 50%; grade 3, deep ulceration with loss of thickness more than 50%; grade 4, full thickness chondral wear with exposure of subchondral bone. A cartilage defect also had to be present in at least two consecutive slices. The highest score at a subregion of each region was used to represent the score of this region. Total cartilage defect scores were obtained by summing the scores of medial and lateral tibial, medial and lateral femoral and patellar sites. Two observers estimated cartilage defects at all sites. Correlation coefficients for

intraobserver reliability and interobserver reliabilities in our hands were 0.89–0.94 and 0.85–0.93, respectively[17].

Cartilage volumes were determined on T1-weighted MRI with image processing on an independent work station using the software program OsiriX. The total cartilage volume was divided into medial tibial, lateral tibial and patellar cartilage volume by manually drawing disarticulation contours around the cartilage boundaries section-by-section, which were then re-sampled for final 3-D rendering. One observer measured cartilage volume. The coefficients of variation (CV) for this method were 2.1–2.6%[1].

BMLs (bone marrow lesions) were defined as discrete area of increased signal adjacent to subcortical bone at the tibia and femur on T2-weight MRI using a semiquantitative (0–3) scoring system (Whole-Organ Magnetic Resonance Imaging Score [WORMS])[15]: grade 0, none; grade 1, no more than 25% of the region; grade 2, 25–50% of the region; grade 3, more than 50% of the region. The highest score at a subregion of each region was used to represent the score of this region. Total BML scores were obtained by summing the scores of medial and lateral tibial, medial and lateral femoral and patellar sites. One observer assessed BMLs. The intraobserver reliabilities ranged between 0.89 and 1.00, as described[18].

IPFP (infrapatellar fat pad) signal intensity alteration on T2-weighted MRI was recorded if hyperintense signal alterations were observed within the IPFP. Signal intensity alteration, defined as discrete areas of increased signal within the IPFP, was graded as follows (MRI Osteoarthritis Knee Score [MOAKS]): grade 0, none; grade 1, less than 10% of the region; grade 2, 10–20% of the region; and grade 3, more than 20% of the region[19]. Two observers graded the IPFP signal intensity alteration. Intraobserver and interobserver reliabilities were assessed in 30 subjects with an intraclass correlation coefficient (ICC) of 0.95 and an ICC of 0.94, respectively.

Effusion-synovitis was assessed as the presence of intraarticular fluid-equivalent signal on sagittal T2-weighted MRI. Effusion synovitis was investigated in the following four regions of interest (ROIs) according to the anatomy of the knee joint synovial cavity[20]. They were: (1) suprapatellar pouch, extends superiorly from the upper surface of the patellar, between the posterior suprapatellar fat pad (quadriceps femoris tendon) and the anterior surface of the femur; (2) central portion, lies between the central femoral and tibial condyles, around the ligaments and menisci; (3) posterior femoral recess, lies behind the posterior portion of each femoral condyle and the deep surface of the lateral and medial heads of the gastrocnemius; (4) subpopliteal recess, lies posteriorly between the lateral meniscus and the popliteal tendon. The size of effusion-synovitis in each of ROIs (cm^2) was directly generated in the entire series of images using OsiriX software. A total area of all ROIs in the same slice was summed as effusion-synovitis area of this slice. A maximal area in one slice was selected to represent effusion-synovitis area of the knee. Two observers measured the maximal area of effusion-synovitis. The intraobserver and interobserver reproducibility was 0.81 and 0.60, respectively[21].

Statistical analysis

Student's *t* tests, chi-squared tests, and Mann–Whitney *U* tests were used to compare means, proportions, and medians, respectively. The residuals for linear regression models of cartilage degradation enzymes with S100A8/S100A9 were not normally distributed, so the Box-Cox transformations for these enzymes were performed. The calculated λ values of MMP3, MMP10 and MMP13 were 0, 0.41 and 1.5; therefore, we did natural log transformation for MMP3, and transformations for MMP10 and MMP13 were calculated by the λ values. Linear regression analyses were used to examine the associations between S100A8/S100A9 (the independent variable) and WOMAC scores, cartilage volumes, effusion-synovitis area and transformed cartilage degradation enzymes before and after adjustment for age, sex and BMI. Ordinal regression analyses were used to examine the associations between S100A8/S100A9 (the independent variable) and K–L grades, JSN, osteophytes, cartilage defects, BMLs and IPFP signal intensity alternation before and after adjustment for age, sex and BMI. The performed regression analyses were based on the assumption that the dependent variable was a function of the independent variables, and thus the estimated associations were directional effects rather than causally undefined relations. Standard diagnostic checks of model fit and residuals were routinely done, and data points with large residuals and/or high influence were investigated for data errors. A P value < 0.05 (two-tailed) or 95% confidence interval (CI) not including the null point (for linear regression) or 1 (for ordinal regression) was considered statistically significant. All statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc, USA).

Results

A total of 141 subjects (84.4% females) aged between 37 and 73 years (mean 55.0 years) were included in the analyses. There were no statistically significant differences in demographic factors (age, sex and BMI) between these participants and those excluded ($n = 64$; data not shown). The median S100A8/S100A9 level was 291.96 ng/mL. Characteristics of the participants are presented in Table I. Patients with higher and lower levels of S100A8/S100A9 (split at the median level) were similar in age, gender, height, weight, BMI, disease duration, occupation, WOMAC score, prevalence of JSN, osteophytes and ROA, cartilage volume, cartilage defects score, BMLs score, effusion-synovitis area, proportions of IPFP signal intensity alteration of ≥ 2, serum levels of MMP3, MMP10 and MMP13.

Associations between serum S100A8/A9 and joint symptoms (WOMAC score) are shown in Table II. Serum levels of S100A8/S100A9 were significantly and positively associated with total WOMAC score before and after adjustment for age, sex and BMI (β: 0.107 per 10 ng/ml, $P = 0.034$; 0.111 per 10 ng/ml, $P = 0.021$, respectively). There was no statistically significant association between serum S100A8/S100A9 and WOMAC pain in univariable and multivariable analyses, but serum S100A8/S100A9 was significantly and positively associated with weight-bearing pain after adjustment for age, sex and BMI (β: 0.015 per 10 ng/ml, $P = 0.043$). Serum levels of S100A8/S100A9 were not significantly associated with WOMAC stiffness before and after adjustment for age, sex and BMI, while serum S100A8/S100A9 had a positively significant association with WOMAC physical dysfunction in univariable and multivariable analyses (β: 0.087 per 10 ng/ml, $P = 0.015$; 0.091 per 10 ng/ml, $P = 0.010$, respectively).

Associations between serum S100A8/A9 and cartilage defects are presented in Table III. Serum S100A8/S100A9 was significantly and positively associated with total cartilage defects and cartilage defects at lateral femoral, lateral tibial and medial femoral sites (ORs: 1.006–1.008 per 10 ng/ml, all $P < 0.05$), but not with cartilage defects at medial tibial and patellar sites before and after adjustment for age, sex and BMI. Associations between serum S100A8/S100A9 and JSN, osteophytes, K–L grades, cartilage volumes, BMLs, effusion-synovitis area and IPFP signal intensity alteration did not reach statistical significance (data not shown).

Associations between serum S100A8/S100A9 and cartilage degradation enzymes are shown in Table IV. Serum S100A8/S100A9 was significantly and positively associated with MMP3 before and after adjustment for age, sex and BMI (β: 0.002 per 10 ng/ml, $P = 0.033$; β: 0.002 per 10 ng/ml, $P = 0.032$, respectively). There was no statistically significant association between serum S100A8/

Table I
Characteristics of participants (split by median level of S100A8/A9)

	S100A8/A9 ≤ median ($n = 71$)	S100A8/A9 > median ($n = 70$)	*P* values
Age, years*	54.45 (8.24)	55.64 (8.06)	0.391
Female sex, %†	84.5	84.3	0.976
Height, cm*	159.16 (5.67)	160.16 (7.91)	0.396
Weight, kg*	65.28 (10.27)	66.40 (10.78)	0.535
BMI, kg/m^2*	25.82 (4.28)	25.81 (3.22)	0.988
Disease duration, months‡	7.5 (2.0, 36.0)	10.0 (2.0, 36.5)	0.812
Occupation, %†			0.694
Manager/Administrator	20.3	24.6	
Professional	26.1	27.5	
Manual labor	52.2	47.8	
Others	1.4	0	
WOMAC score			
Pain*	21.39 (9.33)	22.29 (8.56)	0.553
Stiffness*	7.11 (4.24)	7.19 (4.32)	0.919
Physical dysfunction*	69.47 (31.41)	74.77 (26.26)	0.283
Total*	96.59 (43.31)	102.76 (37.73)	0.369
Osteophytes, %†	84.4	83.9	0.923
JSN, %†	98.4	98.4	>0.999
Knee ROA, %†	68.8	75.8	0.269
Total cartilage volume, cm^3*	4.16 (0.97)	4.488 (1.15)	0.479
Total cartilage defect score*	21.63 (6.80)	21.94 (5.29)	0.787
Total BMLs score*	3.33 (3.47)	4.00 (3.45)	0.318
Effusion-synovitis area, cm^2*	1.58 (1.24)	1.69 (1.56)	0.647
IPFP signal intensity alteration ≥ 2, %†	36.5	40.3	0.581
MMP3, ng/ml‡	0.56 (0.32, 0.76)	0.56 (0.36, 0.79)	0.487
MMP10, ng/ml‡	15.58 (7.27, 27.77)	19.95 (9.29, 33.32)	0.062
MMP13, ng/ml‡	121.88 (65.31, 124.16)	121.20 (60.62, 124.28)	0.606

S100A8/A9 median level 291.96 ng/mL (interquartile range 227.24–458.33).
* *t* tests were used mean (standard deviation).
† χ^2 tests were used for the proportions.
‡ Mann–Whitney *U* tests were used for median (interquartile range).

Table II
Associations between S100A8/A9 and WOMAC score

	Univariable β (95% CI)	*P* value	Multivariable* β (95% CI)	*P* value
Total WOMAC score	**0.107 (0.008, 0.205)**	**0.034**	**0.111 (0.017, 0.204)**	**0.021**
Pain	0.009 (−0.013, 0.031)	0.407	0.010 (−0.011, 0.032)	0.343
Weight-bearing pain	0.015 (0.000, 0.030)	0.050	**0.015 (0.000, 0.030)**	**0.043**
Non-weight-bearing pain	0.005 (−0.006, 0.015)	0.382	0.005 (−0.006, 0.015)	0.365
Stiffness	0.005 (−0.005, 0.016)	0.342	0.006 (−0.005, 0.016)	0.285
Physical dysfunction	**0.087 (0.017, 0.156)**	**0.015**	**0.091 (0.022, 0.159)**	**0.010**

Dependent variables: WOMAC score. Independent variable: S100A8/A9.
The unit of S100A8/A9 used here was "per 10 units".
β: unstandardised regression coefficient.
* Adjusted for age, sex, and BMI. Data in bold denote statistically significant results.

Table III
Associations between S100A8/A9 and cartilage defects

	Univariable OR (95% CI)	*P* value	Multivariable* OR (95% CI)	*P* value
Cartilage defects (total)	**1.006 (1.000, 1.011)**	**0.041**	**1.006 (1.001, 1.012)**	**0.025**
Lateral femoral	**1.007 (1.000, 1.012)**	**0.050**	**1.007 (1.001, 1.013)**	**0.031**
Lateral tibial	**1.007 (1.001, 1.013)**	**0.020**	**1.008 (1.002, 1.014)**	**0.010**
Medial femoral	**1.007 (1.001, 1.013)**	**0.024**	**1.008 (1.002, 1.014)**	**0.015**
Medial tibial	1.001 (0.995, 1.006)	0.838	1.001 (0.995, 1.007)	0.734
Patellar	1.002 (0.996, 1.007)	0.488	1.002 (0.997, 1.008)	0.467

Dependent variables: cartilage defect scores in the respective site and total knee. Independent variable: S100A8/A9.
The unit of S100A8/A9 used here was "per 10 units".
OR: odds ratio.
* Adjusted for age, sex and BMI. Data in bold denote statistically significant results.

S100A9 and MMP10 in univariable analyze, but after adjustment for age, sex and BMI, the association of serum S100A8/S100A9 with MMP10 was of borderline statistical significance (β: 0.046 per 10 ng/ml, $P = 0.053$). We did not find statistically significant associations between serum S100A8/S100A9 and MMP13 before and after adjustment for potential confounders.

Discussion

To the best of our knowledge, this is the first epidemiological study to investigate the associations between serum levels of S100A8/S100A9 and knee symptoms, joint structural changes, and cartilage degradation enzymes in patients with knee OA. We found

Table IV
Associations between S100A8/A9 and cartilage degradation enzymes

	Univariable β (95% CI)	*P* value	Multivariable* β (95% CI)	*P* value
MMP3	**0.002 (0.000, 0.003)**	**0.033**	**0.002 (0.000, 0.003)**	**0.032**
MMP10	0.040 (−0.006, 0.085)	0.088	0.046 (−0.001, 0.096)	0.053
MMP13	1.009 (−0.172, 2.190)	0.093	0.097 (−0.228, 2.182)	0.111

Dependent variables: cartilage degradation enzymes (after Box-Cox transformation). Independent variable: S100A8/A9.
The unit of S100A8/A9 used here was "per 10 units".
β: unstandardised regression coefficient.
* Adjusted for age, sex, and BMI. Data in bold denote statistically significant results.

that after adjustment for age, sex and BMI, serum levels of S100A8/S100A9 were positively associated with total WOMAC score, WOMAC weight-bearing pain and WOMAC physical dysfunction. S100A8/S100A9 was also significantly associated with increased total cartilage defects and cartilage defects at lateral femoral, lateral tibial and medial femoral sites, but was not associated with other joint structural changes. Furthermore, S100A8/S100A9 had a positively significant association with serum MMP3 and a borderline level of statistically significant association with serum MMP10.

Previous studies showed that S100A8/S100A9 was involved in the pathogenesis of OA in CIOA mice[9–11]. S100A8/S100A9 may play roles in OA through different mechanisms. Stimulation of chondrocytes from OA donors with S100A8 and S100A9 could up-regulate catabolic markers (MMPs 1, 3, 9, and 13, interleukin [IL]-6, IL-8, and monocyte chemotactic protein 1) and down-regulate anabolic markers (aggrecan and type II collagen), thereby favoring cartilage breakdown[22]. S100A9 stimulation of OA synovial tissue increased the production of IL-1β, IL-6, IL-8, tumor necrosis factor-α and MMPs probably through stimulating the local macrophages[23]. Intra-articular injection of S100A8 in mice recruited pro-inflammatory monocytes to the joint, and these monocytes were high producers of S100A8/S100A9 and thereby might form a positive feedback loop[24]. However, the findings from clinical research have been inconsistent. In a cohort with early symptomatic OA patients, patients with osteophyte score progression or K–L score progression both had higher serum/plasma levels of S100A8/S100A9 in baseline compared to the non-progressors[8,10]. In contrast, a cross-sectionally exploratory study did not found significant association between serum S100A8/S100A9 and clinical and structural characteristics of patients with OA[25].

The most common symptoms of knee OA are pain, stiffness, and physical dysfunction, which affect the quality of life of OA patients[26]. The WOMAC is the most commonly used disease-specific measure of outcome used in OA, and has 24 items with a total score and three subscales: pain, stiffness and physical function[27]. In our study, S100A8/S100A9 was positively associated with total WOMAC score, WOMAC weight-bearing pain and WOMAC physical dysfunction. These suggested that S100A8/S100A9 could have a link with symptoms in knee OA, but the causal relationship and the underlying mechanisms need to be examined by future longitudinal studies and experimental researches.

Cartilage defects are commonly found in people with knee OA[28]. They can predict subsequent cartilage loss and the need for knee joint replacement, and are associated with other joint structural changes; thereby, cartilage defects could be used as early markers in the development and progression of knee OA[28,29]. In our study, S100A8/S100A9 was associated with increased cartilage defects, suggesting S100A8/S100A9 may play roles in the pathogenesis of OA cartilage defects. Serum S100A8/S100A9 was not significantly associated with cartilage volume and other knee structural changes. The underlying reasons for these inconsistent results are unclear, one possible explanation for this is that S100A8/S100A9 might only have a role in early articular cartilage damage (as S100A8/S100A9 does not have a sustained role in cartilage degradation in experimental OA)[25], and cartilage defects occur prior to cartilage volume lose (as cartilage defects can predict cartilage loss, and may instigate the development of early OA-like degenerative changes in the articular cartilage)[29,30]. Knee cartilage defects were significantly associated with knee pain and contributed to an impaired function of the knee[29,30]. Whether this can explain the positive associations between serum S100A8/S100A9 and knee symptoms in this study needs to be examined by further studies.

MMPs were proved to induce cartilage degradation and promote the development of OA[2]. Among these MMPs, MMP3 is responsible for disrupting the collagen cross link of telopeptide of type-II and type IX collagens resulting in disruption of fiber structure and function[31]; MMP10 has a structure and substrate specificity similar to that of MMP3 and can activate procollagenases that are relevant to cartilage degradation[32]; and MMP13 is an enzyme which plays an important role in type II collagen degradation in articular cartilage[33]. We found that S100A8/S100A9 was positively associated with serum MMP3, and also had a trend of positive associations with MMP10 and MMP13. This result suggested that S100A8/S100A9 may play roles in cartilage defect by up-regulating the expression of these MMPs.

There are several limitations in this study. First, it was a cross-sectional study so causalities between the risk factor and outcomes in this study could not be established. Further longitudinal studies are needed to verify our findings. Second, the participants were recruited from the clinics consecutively rather than being selected from the community randomly; therefore, the results may not be generalizable to the community-based knee OA patients. Third, 64 patients were excluded from this study which may cause selection bias because these patients may be different in characteristics with those included. However, there were no significant differences in demographic factors between those included and excluded, suggesting the selection bias may not exist. Fourth, the sample size was relatively small, and it is possible that with a larger sample size, more significant associations can be detected. Fifth, we did not record deformities, misalignment, post-traumatic knee injury and end-stage knee OA in the current study. These factors may confound the associations of S100A8/S100A9 with knee OA measures and further studies are required to clarify this. Last, the levels of S100A8/S100A9 and cartilage degradation biomarkers were measured in serum rather than in synovial fluid, so their local effects were unknown.

In conclusion, serum levels of S100A8/S100A9 were positively associated with increased knee symptoms, cartilage defects and serum cartilage degradation enzymes in patients with knee OA, suggesting that S100A8/S100A9 may have a role to play in knee OA. Future longitudinal studies are required to confirm the findings. Overall, combining with findings from previous studies, our results suggest that S100A8/S100A9 may play a detrimental role in OA articular cartilage metabolism and could serve as a serum biomarker as well as a therapeutic target of knee OA in the future.

Author contributions

Study conception and design: CD and JX.
Acquisition of data: KW, JW and GR.
Analysis and interpretation of data: all authors.
Drafting of the article: GR and CD.
Revising and final approval of the article: All authors.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this study.

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