

ORIGINAL ARTICLE

Decreased Synovial Fluid SLC7A11 and GPX4 Levels Correlate with Knee Osteoarthritis Disease Severity

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ABSTRACT

Background: Ferroptosis has been suggested to play a role in the pathogenesis of primary knee osteoarthritis (OA). The current study aimed to explore the potential relationship between the concentrations of SLC7A11 and GPX4 in serum and synovial fluid (SF) and the severity of knee OA.

Methods: A total of 120 patients with knee OA and 120 healthy controls were included in this study. Serum and SF levels of SLC7A11 and GPX4 were measured using ELISA before and after a comprehensive therapeutic intervention. Radiographic severity of OA was evaluated using the Kellgren-Lawrence (K-L) grading system. Clinical severity was assessed via Visual Analogue Scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores. Levels of interleukin-6 (IL-6) and matrix metalloproteinase-3 (MMP-3) were also analyzed. Receiver operating characteristic (ROC) curve analysis was employed to evaluate the diagnostic utility of SLC7A11 and GPX4 levels in relation to K-L grades.

Results: Serum levels of SLC7A11 and GPX4 did not show significant differences between knee OA patients and healthy controls. However, patients with K-L grade 4 exhibited statistically significant lower concentrations of SLC7A11 and GPX4 in SF compared to those with K-L grades 2 and 3. Furthermore, SF levels of these proteins were significantly lower in patients with K-L grade 3 compared to those with grade 2. Negative correlations were observed between SF levels of SLC7A11 and GPX4 and K-L grades, as well as with SF levels of IL-6 and MMP-3. SF concentrations of SLC7A11 and GPX4 were also inversely correlated with VAS and WOMAC scores. ROC analysis indicated that both SLC7A11 and GPX4 demonstrated good diagnostic value for assessing radiographic progression in knee OA.

Conclusions: These results indicate that SLC7A11 and GPX4 may function as potential biomarkers for monitoring disease progression and therapeutic efficacy in knee osteoarthritis.
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KEYWORDS

knee osteoarthritis, ferroptosis, SLC7A11, GPX4, disease severity

INTRODUCTION

Knee osteoarthritis (OA) is a widespread degenerative joint disorder that substantially diminishes the quality of life for millions globally. The disease is marked by the progressive breakdown of articular cartilage, resulting in pain, stiffness, and impaired joint function [1]. The

underlying mechanisms of knee OA are complex, involving an interplay of genetic predisposition, mechanical stress, and inflammatory processes [2]. It is estimated to affect over 23% of the global population aged 40 and above [3]. In China, a recent population-based longitudinal study reported an overall symptomatic knee OA prevalence of 14.6%, with a higher incidence in women (19.1%) compared to men (10.9%) [4]. The rising annual number of total knee arthroplasty procedures underscores both the significant healthcare burden and the current lack of treatments capable of arresting disease progression [5]. Existing pharmacological approaches for knee OA primarily focus on alleviating symptoms. Given the escalating prevalence of the condition, key therapeutic goals include preventing structural deterioration and managing symptoms or functional impairment. These objectives are also central to the development of disease-modifying osteoarthritis drugs (DMOADs) [5].

The diagnosis of primary knee osteoarthritis (OA) primarily depends on radiographic findings and patient-reported symptoms, such as pain and functional impairment [6]. However, radiographic features like osteophyte formation and joint space narrowing are generally indicative of moderate to advanced stages of the disease [7]. Identifying reliable biomarkers for knee OA is essential for early detection, tracking disease progression, and assessing treatment efficacy [8]. Recent research has emphasized the promise of various biochemical biomarkers in elucidating and forecasting the course of knee OA [9].

Ferroptosis is a regulated form of cell death driven by iron-dependent lipid peroxidation [10], and it has attracted considerable interest for its involvement in numerous pathological conditions, including cancer [11], cardiovascular disorders [12], and immune-related diseases [13], among others. Key regulators of ferroptosis include solute carrier family 7 member 11 (SLC7A11) and glutathione peroxidase 4 (GPX4), which constitute a vital axis in modulating cellular antioxidant defense mechanisms [14]. SLC7A11, also referred to as xCT, is an essential component of the cystine/glutamate antiporter system. It plays a pivotal role in preserving cellular redox balance by enabling cystine uptake for glutathione synthesis [15]. This process is critical for shielding cells from oxidative stress and controlling ferroptosis, a programmed cell death pathway marked by iron-dependent lipid peroxidation. SLC7A11 mediates cystine import, which is necessary for producing glutathione (GSH), a major antioxidant. Conversely, GPX4 functions to reduce lipid peroxides, thereby averting the oxidative damage that triggers ferroptosis [16]. The interaction between these two molecules is vital for sustaining cellular redox equilibrium and preventing ferroptotic cell death [17].

Within cartilage and the context of osteoarthritis (OA), the role of GPX4 gains further importance as it participates in alleviating chondrocyte ferroptosis, a key contributor to cartilage breakdown and OA advancement.

The control of GPX4 and its effects on chondrocyte survival and cartilage integrity are shaped by multiple signaling pathways and molecular interactions. For example, the RNA-binding protein SND1 has been demonstrated to accelerate GPX4 degradation by destabilizing HSPA5 mRNA, thereby increasing ferroptosis in osteoarthritic chondrocytes [18]. Likewise, excessive mechanical loading can trigger GPX4-mediated chondrocyte ferroptosis through the Piezo1 channel, indicating that mechanical stress is a significant contributor to OA pathogenesis [19]. These observations highlight the intricate regulatory networks that govern GPX4 activity and their consequences for cartilage health.

The regulation of SLC7A11 is also particularly relevant to cartilage homeostasis and disease, including OA. SLC7A11 expression is essential for maintaining chondrocyte viability by inhibiting ferroptosis. Research has indicated that targeting SLC7A11 can adjust ferroptosis and consequently affect OA progression [20]. Furthermore, the Nrf2 signaling pathway, activated by various agents like curcumin and ruscogenin, can enhance the expression of SLC7A11 and GPX4, thus conferring protection against ferroptosis in chondrocytes and presenting therapeutic opportunities for OA [21,22].

Collectively, the aforementioned studies suggest that SLC7A11 and GPX4 likely play significant roles in safeguarding knee OA cartilage from damage. Nevertheless, no investigations have yet examined the levels of SLC7A11 and GPX4 in the serum and synovial fluid (SF) of knee OA patients. Therefore, we measured the concentrations of SLC7A11 and GPX4 in serum or SF from knee OA patients and evaluated their correlation with disease severity to investigate whether ferroptosis may influence the pathophysiological mechanisms underlying knee OA.

MATERIALS AND METHODS

Study subjects

This study enrolled 240 participants, comprising 120 patients diagnosed with knee osteoarthritis (OA) and 120 age- and gender-matched healthy controls undergoing routine health examinations. Recruitment took place at our hospital between September 2024 and December 2025. The diagnosis of knee OA in the patient group was established based on the criteria set forth by the American College of Rheumatology [23]. Inclusion criteria for the OA cohort were: 1) a confirmed diagnosis of primary knee OA; 2) age over 18 years; and 3) radiographic confirmation of OA, classified as Kellgren-Lawrence (K-L) grade 2 to 4. Individuals with a history or evidence of rheumatoid arthritis, gout, traumatic arthritis, or other immune-mediated diseases were excluded. The study protocol received approval from the Institutional Review Board of Zhuhai Hospital of Integrated Traditional Chinese & Western Medicine (ethical no. 2025-03-023-F01). All patients were informed about the aim of the study.

ELISA analysis

Fasting blood samples were collected from all participants in the morning. Synovial fluid (SF) samples were aspirated from the most symptomatic knee. Serum and SF were separated by centrifugation at $3,000 \times g$ for 10 minutes and aliquoted into 1.5 mL Eppendorf tubes. Serum and SF samples were processed within 2 hours of arthrocentesis and were subsequently transferred to a -80°C freezer for long-term storage within 30 minutes of aliquoting. All samples were aliquoted immediately upon processing to avoid repeated freeze-thaw cycles. Each aliquot was thawed only once for the analysis. The concentrations of SLC7A11 (cat no. SEE410Hu, Cloud-clone Inc., Katy, TX, USA) and GPX4 (cat no. SEE99-4Hu, Cloud-clone Inc., Katy, TX, USA) in serum and SF were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits, strictly adhering to the manufacturer's instructions. In detail, the sample dilution factor was 1:10 for tissue homogenates. The standard curve was prepared using the manufacturer's provided standards in a serial two-fold dilution across seven points; plate washing was performed three times with 300 μL of the supplied wash buffer using an automated plate washer; and incubations were carried out for 90 minutes at 37°C for the capture step and 60 minutes at room temperature for the detection step, as per the kit protocol. Absorbance was measured at 450 nm using a microplate reader. The detection ranges were 0.156 - 10 ng/mL for SLC7A11 and 3.12 - 200 ng/mL for GPX4. The intra-assay and inter-assay coefficients of variation were 6.8% and 7.5% for SLC7A11 and 8.8% and 9.3% for GPX4, respectively. Synovial fluid IL-6 (cat no. ab178013, Abcam, Cambridge, UK) and MMP-3 levels were also detected (cat no. ZN2327-XZA, Biolab, Beijing, China). The detection ranges were 7.8 - 500 pg/mL for IL-6 and 156 pg/mL - 10,000 pg/mL for MMP-3. The intra-assay and inter-assay coefficients of variation were 5.3% and 6.5% for IL-6 and 6.6% and 9.1% for MM-3, respectively. The reported intra- and inter-assay CVs were derived from our in-house validation experiments. The validation was performed by analyzing replicates of pooled control samples within the same assay plate (intra-assay, $n = 8$) and across three independent assay runs on different days (inter-assay, $n = 3$). Each patient sample was measured in triplicate (three technical replicates) within a single experimental run.

Assessment of radiographic severity

The radiographic severity of knee OA was evaluated using the Kellgren-Lawrence (K-L) grading system [24], a widely recognized tool for this purpose. This system assesses degenerative joint changes visible on standard anteroposterior weight-bearing knee radiographs, including osteophyte formation, joint space narrowing, and subchondral sclerosis, to categorize the extent of structural damage. The grading is defined as follows: Grade 0: No radiographic abnormalities; Grade 1: Doubtful joint space narrowing or minimal osteophyte

formation, typically with minimal or no symptoms; Grade 2: Definite small osteophytes and possible joint space narrowing, often associated with activity-related pain; Grade 3: Moderate osteophytes, definite joint space narrowing, and subchondral sclerosis, frequently causing pain and functional impairment; and Grade 4: Large osteophytes, severe joint space narrowing, marked sclerosis, and possible bone deformity, associated with severe pain and significant disability. For this study, patients with a K-L grade of 2 or higher were included. In cases of bilateral involvement, the knee with the higher K-L grade was selected for analysis. All radiographic assessments were performed independently by two experienced radiologists, and inter-observer agreement was quantified using the Kappa statistic. Disagreements in K-L grading between the two radiologists were resolved through a consensus discussion. In detail, following their independent initial assessments, the two radiologists jointly reviewed the cases where their grades differed. They discussed the radiographic features until a mutual agreement was reached on the final K-L grade for each discrepant case. This consensus process ensured a single, definitive grade was used for all subsequent analyses.

Assessment of clinical severity

Clinical symptom severity and physical function were assessed using patient-reported outcome measures. Pain intensity was evaluated with a Visual Analog Scale (VAS) (0 - 10 cm). Overall disease impact was measured using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [25]. The WOMAC index consists of 24 items across three subscales: pain (5 items), stiffness (2 items), and physical function (17 items). Total scores range from 0 to 96, with higher scores indicating worse pain, stiffness, and functional limitations. The WOMAC is a validated, reliable, and widely used instrument in OA clinical research [26].

Comprehensive treatment procedure

The skin over the treatment area was disinfected three times. Each patient was lying in supine position with their knees slightly flexed and supported on a small pillow. All patients received sodium hyaluronate injection into the knee joint once per week. The dose used was 2.5 mL per knee per injection, containing 25 mg of sodium hyaluronate (molecular weight 1.5 - 2.0 million Daltons, HailiDa, China). Acupuncture and massage were applied 3 times/week for consecutive 4 weeks. In detail, the primary acupuncture points used were ST35 (Dubi), EX-LE5 (Xiyan), GB34 (Yanglingquan), SP10 (Xuehai), and ST36 (Zusanli). Sterile, single-use stainless steel needles (0.25 mm in diameter, 40 mm in length) were used. The insertion depth ranged from 10 to 30 mm depending on the point and the patient's body type. Manual stimulation was applied to achieve the "deqi" sensation, with needles retained for 30 minutes per session. The technique used was a standardized Chinese therapeutic massage (Tui Na) focusing on the knee

joint and surrounding musculature, including techniques such as rolling, kneading, and pressing. The duration of each massage session was 20 minutes. All massage treatments were administered by licensed physical therapists with a minimum of 5 years of clinical experience in musculoskeletal rehabilitation, who underwent a specific training session for protocol standardization prior to the study. The SF was extracted at week one and week 4 following treatment to examine the change of SLC7A11 and GPX4 levels.

Statistical analysis

All data are expressed as mean $\pm$ standard deviation (SD) derived from three independent experiments. For comparisons between two groups, Student's *t*-test was employed. Comparisons among three or more groups were conducted using one-way analysis of variance (ANOVA), followed by the Bonferroni post-hoc test for selected pairwise comparisons. For comparison of GPX4 and SLC7A11 levels between baseline, week one, and week 4, repeated measure analysis of variance was utilized. All statistical analyses were performed using GraphPad Prism software (version 8.0). Receiver operating characteristic (ROC) curve analysis was utilized to assess the diagnostic potential of GPX4 and SLC7A11 in relation to the Kellgren-Lawrence (K-L) grade. Spearman's correlation coefficient was calculated to evaluate potential associations between GPX4 and SLC7A11 level with K-L grade. Pearson's correlation coefficient was used to assess the correlation of GPX4 and SLC7A11 level with other indices. A *p*-value of less than 0.05 was considered indicative of statistical significance.

Post-hoc statistical power calculation

Statistical power ($1-\beta$) was calculated using PASS (Power Analysis and Sample Size) 2008 Statistical Software (UT, USA) using the formula below according to the obtained data of mean GPX4 or SLC7A11 levels, standard error, enrolled numbers of patients in each group. Statistical power was regarded strong when > 0.8 . The detailed calculation formula is listed below:

$$1 - \beta = \Phi(z - z_{1-\alpha/2}) + \Phi(-z - z_{1-\alpha/2})$$

$$z = (\mu_A - \mu_B) / \sigma \left(\frac{1}{n_A} + \frac{1}{n_B} \right)$$

RESULTS

Demographic statistics

Basic characteristics between primary knee OA patients and healthy controls

The baseline demographic and clinical characteristics of the study participants are summarized in Table 1. The statistical power was 0.95 after calculation. The study cohort comprised 120 patients diagnosed with primary knee OA, aged between 55 and 75 years (mean age:

65.4 $\pm$ 6.5 years), and 120 age-matched healthy controls, aged between 54 and 74 years (mean age: 65.6 $\pm$ 6.6 years). No statistically significant differences were observed between the two groups regarding age, gender distribution, or body mass index (BMI). Furthermore, serum concentrations of SLC7A11 and GPX4 did not differ significantly between OA patients and healthy controls (SLC7A11: 8.1 $\pm$ 2.6 ng/mL vs. 8.3 $\pm$ 2.2 ng/mL, *p* = 0.753; GPX4: 16.4 $\pm$ 3.3 ng/mL vs. 16.5 $\pm$ 3.5 ng/mL, *p* = 0.809). Notably, the levels of both SLC7A11 and GPX4 were significantly lower in SF samples compared to corresponding serum samples from OA patients (SLC7A11: 3.6 $\pm$ 1.3 ng/mL vs. 8.1 $\pm$ 2.6 ng/mL, *p* < 0.001; GPX4: 12.3 $\pm$ 3.8 ng/mL vs. 16.5 $\pm$ 3.5 ng/mL, *p* < 0.001) (Table 1).

SF SLC7A11 and GPX4 levels in knee OA patients with different K-L grades

To explore the association between SF concentrations of SLC7A11 and GPX4 and radiographic disease severity, OA patients were categorized into three subgroups according to the K-L grading system: grade 2 (*n* = 42), grade 3 (*n* = 40), and grade 4 (*n* = 38). The inter-rater reliability for K-L grading between two experienced radiologists was excellent, with a kappa value of 0.98.

Comparative analysis revealed that patients with K-L grade 4 OA exhibited significantly lower SF levels of both SLC7A11 and GPX4 compared to those with grade 3 OA (SLC7A11: 2.5 $\pm$ 0.9 ng/mL vs. 3.8 $\pm$ 1.3 ng/mL, *p* = 0.001; GPX4: 9.5 $\pm$ 2.9 ng/mL vs. 12.2 $\pm$ 3.4 ng/mL, *p* < 0.001) (Figure 1A, 1B). Furthermore, SF concentrations of these markers were also significantly lower in patients with K-L grade 3 OA relative to those with grade 2 OA (SLC7A11: 3.8 $\pm$ 1.3 ng/mL vs. 4.5 $\pm$ 1.0 ng/mL, *p* = 0.004; GPX4: 12.2 $\pm$ 3.4 ng/mL vs. 15.1 $\pm$ 2.7 ng/mL, *p* < 0.001) (Figure 1A, 1B). Subsequent correlation analysis demonstrated a significant inverse relationship between SF levels of SLC7A11 and GPX4 and the K-L grade (SLC7A11: *r* = -0.600, *p* < 0.001; GPX4: *r* = -0.607, *p* < 0.001) (Figure 1C, 1D). In contrast, no significant correlation was found between serum levels of these proteins and the K-L grade (SLC7A11: *r* = 0.002, *p* = 0.613; *r* = -0.014, *p* = 0.597). These correlations still remain significant after adjusted for age and BMI (Tables 2 and 3).

ROC curve analysis

As illustrated in Figure 2, diminished levels of synovial fluid SLC7A11 demonstrated significant differentiating capacity, with notable area under the curve (AUC) values at both the early and medium-to-late stages of knee osteoarthritis (OA). Specifically, for SF SLC7A11, the AUC was 0.672 (*p* = 0.007) when distinguishing between K-L grade 2 and grade 3 (cutoff value: 4.3; sensitivity %: 60.0%; specificity %: 61.9%; 95% confidence interval: 0.553 - 0.790), and 0.763 (*p* < 0.001) for distinguishing between K-L grade 3 and grade 4 (cutoff value: 3.2; sensitivity %: 76.3%; specificity %: 65%; 95% confidence interval: 0.659 - 0.867). Concurrently,

Table 1. Basic characteristics between patients with primary knee OA and healthy controls.

	Knee OA patients (n = 120)	Healthy controls (n = 120)	p-value
Age (years)	65.4 ± 6.5	65.6 ± 6.6	0.626
Gender distribution (F/M)	75/45	72/48	0.813
BMI (kg/m ²)	24.6 ± 3.2	23.8 ± 3.7	0.377
VAS scores	4.9 ± 1.9	/	
WOMAC scores	13.8 ± 4.7	/	
K-L grade (2/3/4)	42/40/38		
Serum SLC7A11 levels ng/mL)	8.1 ± 2.6	8.3 ± 2.2	0.753
SF SLC7A11 levels (ng/mL)	3.6 ± 1.3	/	/
Serum GPX4 levels (ng/mL)	16.4 ± 3.3	16.5 ± 3.5	0.809
SF GPX4 levels (ng/mL)	12.3 ± 3.8	/	

Table 2. Correlations of SF SLC7A11 levels with anthropometric parameters, K-L grades, VAS scores, and WOMAC scores in knee OA patients adjusted for age and BMI.

	SF SLC7A11 levels (ng/mL)		SF SLC7A11 levels (ng/mL) *	
Variables	r	p	r	p
BMI	0.026	> 0.05	/	/
Age	0.104	> 0.05	/	/
K-L grades	-0.600	< 0.001	-0.570	< 0.001
VAS scores	-0.528	< 0.001	-0.487	0.002
WOMAC scores	-0.544	< 0.001	-0.501	0.001

* Adjusted for age and BMI.

Table 3. Correlations of SF GPX4 levels with anthropometric parameters, K-L grades, VAS scores, and WOMAC scores in knee OA patients adjusted for age and BMI.

	SF GPX4 levels (ng/mL)		Serum GPX4 levels (ng/mL) *	
Variables	r	p	r	p
BMI	0.045	> 0.05	/	/
Age	0.109	> 0.05	/	/
K-L grades	-0.607	< 0.001	-0.575	< 0.001
VAS scores	-0.588	< 0.001	-0.543	< 0.001
WOMAC scores	-0.598	< 0.001	-0.560	< 0.001

* Adjusted for age and BMI.

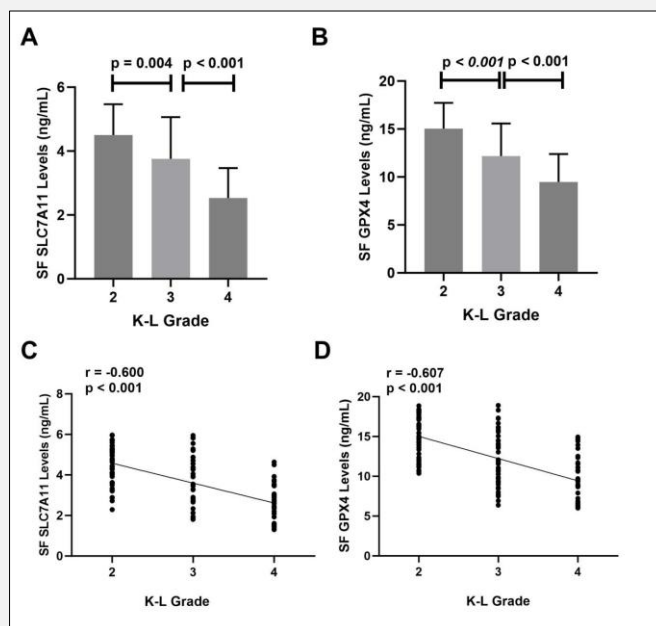


Figure 1. A: Comparison of SF SLC7A11 levels among different K-L grades. **B:** Comparison of SF GPX4 levels among different K-L grades. **C:** Correlation of SF SLC7A11 levels with K-L grades. **D:** Correlation of SF GPX4 levels with K-L grades.

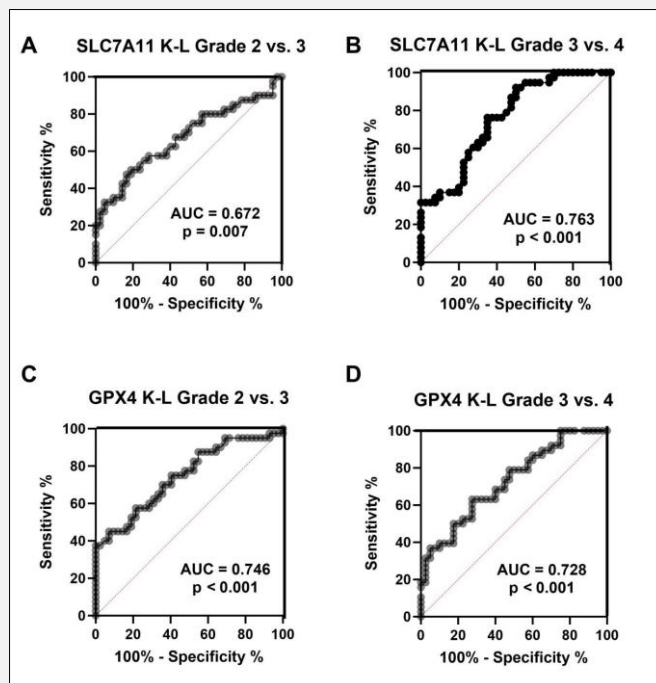


Figure 2. A: ROC curve analysis of SF SLC7A11 levels among K-L grade 2 vs. 3. **B:** ROC curve analysis of SF SLC7A11 levels among K-L grade 3 vs. 4. **C:** ROC curve analysis of SF GPX4 levels among K-L grade 2 vs. 3. **D:** ROC curve analysis of SF GPX4 levels among K-L grade 3 vs. 4.

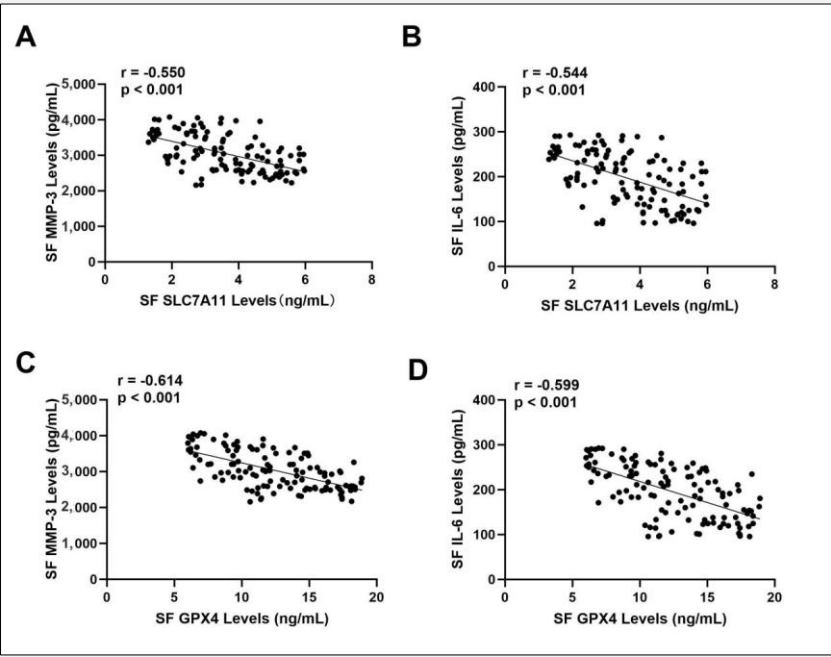


Figure 3. A: Association of SF SLC7A11 levels with SF MMP3 levels. **B:** Association of SF SLC7A11 levels with SF IL-6 levels. **C:** Association of SF GPX4 levels with SF MMP3 levels. **D:** Association of SF GPX4 levels with SF IL-6 levels.

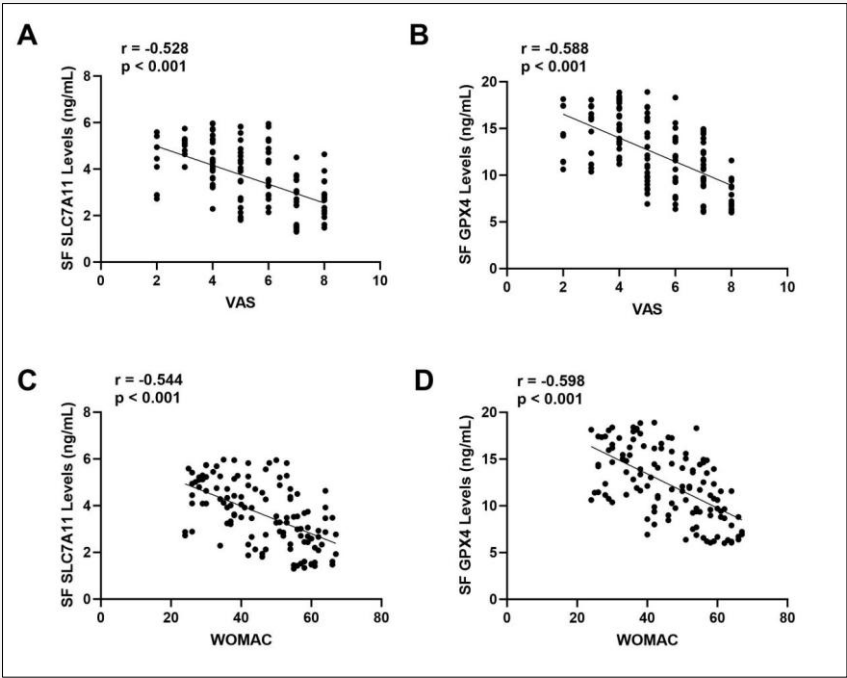


Figure 4. A: Correlation of SF SLC7A11 levels with VAS score. **B:** Correlation of SF GPX4 levels with VAS score. **C:** Correlation of SLC7A11 levels with WOMAC score. **D:** Correlation of GPX4 levels with WOMAC score.

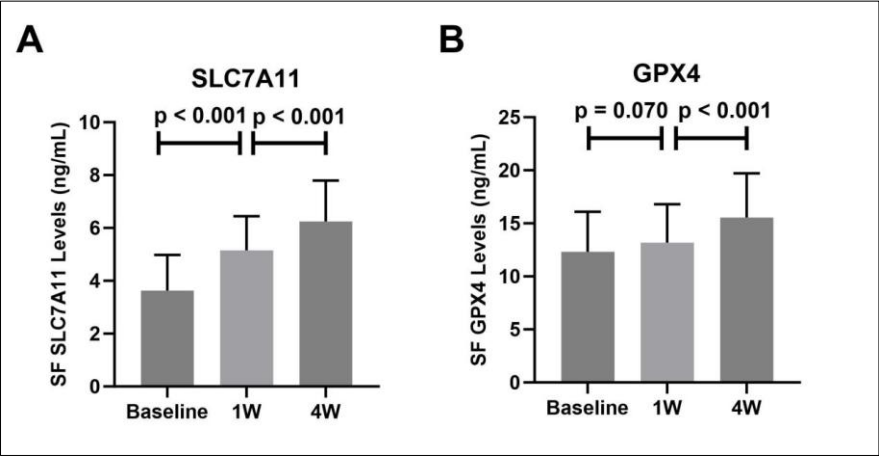


Figure 5. A: Comparison of SF SLC7A11 levels among baseline, week one, and week 4. B: Comparison of SF GPX4 levels among baseline, week one, and week four.

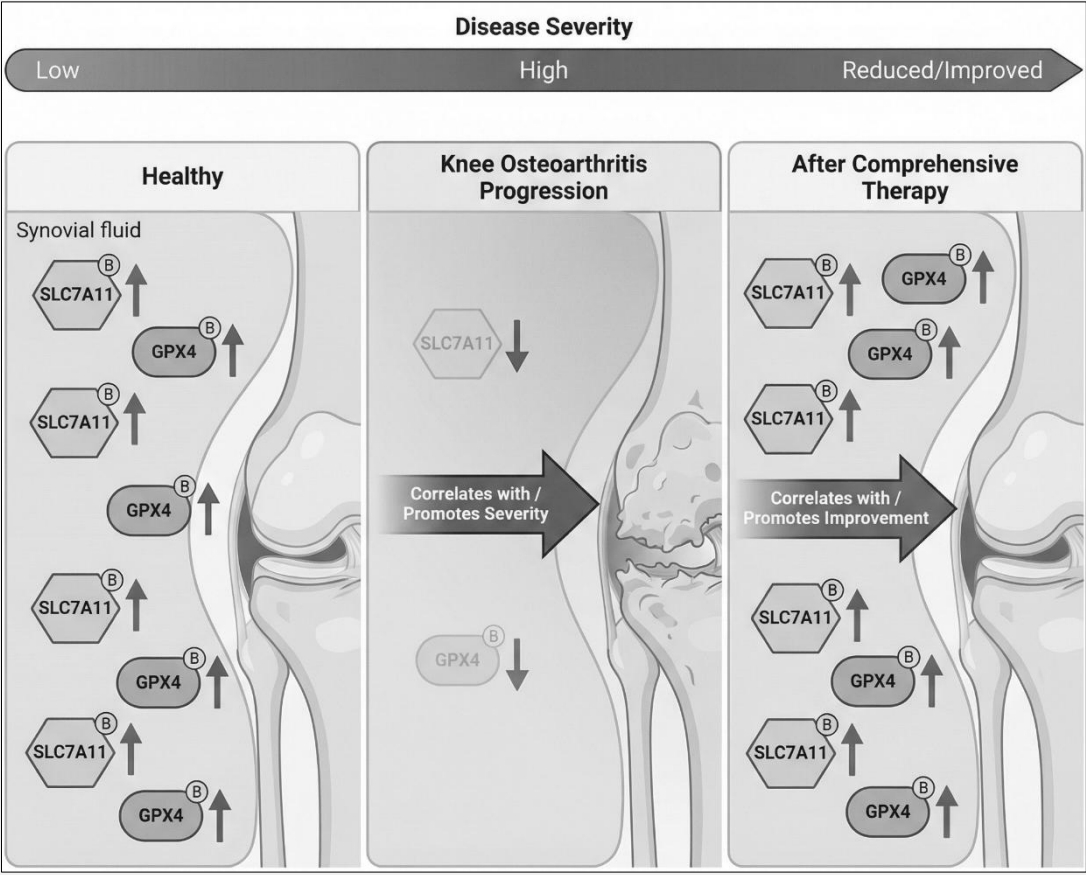


Figure 6. Potential mechanism of the current study.

reduced levels of SF GPX4 also exhibited significant AUC values at these stages: 0.746 ($p = 0.001$) for K-L grade 2 vs. 3 (cutoff value: 14.2; sensitivity %: 70.0%; specificity %: 64.3%; 95% confidence interval: 0.640 - 0.852) and 0.728 ($p < 0.001$) for K-L grade 3 vs. 4 (cutoff value: 9.7; sensitivity %: 63.2%; specificity %: 73.0%; 95% confidence interval: 0.618 - 0.838). These results suggest that decreased concentrations of SLC7A11 and GPX4 in synovial fluid may function as potential biomarkers for monitoring radiographic progression throughout the entire course of knee OA.

Association of synovial fluid SLC7A11 and GPX4 levels with expression of cartilage damage and inflammation markers

Subsequently, we investigated the levels of synovial fluid MMP-3 and IL-6 and their potential relationships with SLC7A11 and GPX4. Intriguingly, a significant inverse correlation was observed between synovial fluid SLC7A11 levels and both MMP-3 ($r = -0.550$, $p < 0.001$; Figure 3A) and IL-6 concentrations ($r = -0.544$, $p < 0.001$; Figure 3B). Similarly, synovial fluid GPX4 levels were also negatively correlated with MMP-3 ($r = -0.614$, $p < 0.001$; Figure 3C) and IL-6 levels ($r = -0.599$, $p < 0.001$; Figure 3D).

Association of synovial fluid SLC7A11 and GPX4 levels with symptomatic severity

We further explored the potential link between synovial fluid SLC7A11 and GPX4 levels and the severity of clinical symptoms in knee OA, as assessed by the Visual Analogue Scale (VAS) and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Our analysis revealed that both synovial fluid SLC7A11 and GPX4 levels were inversely correlated with VAS scores (SLC7A11: $r = -0.528$, $p < 0.001$; GPX4: $r = -0.588$, $p < 0.001$; Figure 4A, B). Furthermore, synovial fluid levels of both proteins showed a significant negative association with the WOMAC index (SLC7A11: $r = -0.544$, $p < 0.001$; GPX4: $r = -0.598$, $p < 0.001$; Figure 4C, D). These correlations still remain significant after adjusted for age and BMI (Tables 2 and 3).

Synovial fluid SLC7A11 and GPX4 levels were increased following comprehensive therapy

Finally, we administered a comprehensive therapeutic regimen to investigate whether synovial fluid SLC7A11 and GPX4 levels would increase post-treatment. Our findings indicated an upward trend in the concentrations of both proteins following therapy.

Synovial fluid SLC7A11 levels were significantly elevated at week one post-treatment compared to baseline (5.2 ± 1.3 ng/mL vs. 3.6 ± 1.3 ng/mL, $p < 0.001$). Moreover, a further substantial increase in SLC7A11 concentration was observed at four weeks post-treatment compared to the week one measurement (6.2 ± 1.6 ng/mL vs. 5.2 ± 1.3 ng/mL, $p < 0.001$; Figure 5A). Regarding GPX4, although the difference between baseline and week one levels did not reach statistical significance

(13.2 ± 3.6 ng/mL vs. 12.3 ± 3.8 ng/mL, $p = 0.07$), its concentration was significantly higher at four weeks compared to both the week one and baseline time points (15.5 ± 4.2 ng/mL vs. 13.2 ± 3.6 ng/mL, $p < 0.001$; 15.5 ± 4.2 ng/mL vs. 12.3 ± 3.8 ng/mL, $p < 0.001$; Figure 5B).

DISCUSSION

Knee osteoarthritis (OA) represents a prevalent degenerative joint disorder predominantly affecting middle-aged and elderly populations, characterized by the progressive deterioration of articular cartilage, osteophyte formation, and synovial inflammation. The multifactorial pathogenesis of OA involves biomechanical stress, inflammatory mediators, and metabolic alterations that culminate in joint pain, stiffness, and functional impairment. This chronic condition imposes significant individual morbidity and substantial socioeconomic burdens globally, driven by increased healthcare utilization and loss of productivity. Despite advances in pharmacologic and non-pharmacologic interventions, current therapeutic options remain limited by suboptimal efficacy and adverse effects, underscoring the urgent need for novel biomarkers to facilitate early diagnosis and monitor disease progression.

This is a cross-sectional study with a simple before-after treatment prospective component. We investigated the concentrations of SLC7A11 and GPX4 proteins in both serum and synovial fluid of patients with knee OA, exploring their associations with disease severity as evaluated by radiographic and clinical assessments. By recognizing the emerging role of ferroptosis, an iron-dependent regulated cell death pathway, in OA pathophysiology, our work aimed to elucidate the relevance of these ferroptosis-related proteins as potential biomarkers. By integrating biochemical measurements with validated clinical scoring systems, we provide comprehensive insights into the involvement of SLC7A11 and GPX4 in OA progression. These findings offer promising avenues for enhancing diagnostic precision and therapeutic monitoring in knee OA management.

The observed differential expression of serum and synovial fluid SLC7A11 and GPX4 in knee osteoarthritis (OA) patients, particularly the significant decline in SF levels correlated with higher Kellgren-Lawrence (K-L) grades, underscoring the involvement of ferroptosis-related pathways in OA pathogenesis. Mechanistically, SLC7A11 functions as a cystine/glutamate antiporter critical for maintaining intracellular glutathione (GSH) pools, while GPX4 enzymatically reduces lipid peroxides, preventing ferroptotic cell death. The depletion of these proteins likely reflects enhanced lipid peroxidation and iron-dependent cell death within the joint microenvironment, contributing to chondrocyte loss and cartilage degradation. This aligns with findings from α -ketoglutarate's protective role in OA via upregulation of the ETV4/SLC7A11/GPX4 axis, which mitigates fer-

roptosis and preserves cartilage matrix integrity [27]. Additionally, the negative correlation between these biomarkers and radiographic severity parallels the diminished anti-ferroptotic defenses observed in non-traumatic osteonecrosis of the femoral head, where reduced local and systemic GPX4 and SLC7A11 levels accompany disease progression [28]. These convergent lines of evidence support the hypothesis that a compromised SLC7A11/GPX4 axis facilitates ferroptosis-driven joint tissue damage in advanced OA stages, highlighting a mechanistic link between ferroptosis and structural deterioration.

The interplay between ferroptosis inhibition and therapeutic intervention emerges from the observed post-treatment restoration of synovial fluid SLC7A11 and GPX4 concentrations. Integrated therapeutic modalities, potentially encompassing physical modalities and pharmacologic agents, may alleviate oxidative stress and restore redox homeostasis within the joint milieu. This is consistent with the cardioprotective effects of dexmedetomidine, which enhances SLC7A11 and GPX4 expression to inhibit ferroptosis in ischemia-reperfusion injury [29], suggesting a conserved mechanism where therapeutic modulation of this axis confers cytoprotection.

Moreover, combined physical therapies demonstrated sustained symptom relief and functional improvements in knee OA, indirectly implicating that such interventions may downregulate ferroptotic pathways, as indicated by reduced WOMAC scores following hybrid treatments [30]. The elevation of SLC7A11 and GPX4 post-treatment in our study thus provides direct biochemical evidence supporting the hypothesis that comprehensive management strategies can reverse ferroptosis-related molecular derangements, potentially translating into clinical benefit.

In terms of prognostic utility, the significant inverse correlations of serum and synovial SLC7A11 and GPX4 levels with clinical indices including VAS and WOMAC scores suggest these biomarkers reflect not only structural but also symptomatic disease burden. This dual association may be underpinned by the role of ferroptosis in amplifying local inflammatory cascades and nociceptive sensitization, as excessive lipid peroxidation products can activate synovial macrophages and induce pro-inflammatory cytokines. This is corroborated by studies demonstrating that early metabolic alterations, including redox imbalances involving glutathione depletion mediated by SLC7A11 and GPX4 dysfunction, exacerbate type 2 inflammatory responses in airway epithelia [31], implying a broader mechanistic paradigm wherein ferroptosis contributes to inflammation-driven symptomatology. Furthermore, the ROC curve analysis establishing the diagnostic sensitivity and specificity of these biomarkers parallels the identification of serum hyaluronan and urinary C2C-HUSA as predictors of OA progression [32], reinforcing the clinical relevance of integrating ferroptosis markers into prognostic models. Collectively, these findings advance the under-

standing of SLC7A11 and GPX4 as molecular correlates of both disease severity and symptomatology in OA.

The patient demographic characteristics, notably age and gender distribution, likely influence the expression and regulation of SLC7A11 and GPX4, thereby modulating disease trajectory. Age-related declines in antioxidant capacity and glutathione metabolism may predispose chondrocytes to ferroptotic susceptibility, as suggested by the gender-specific biomarker profiles identified in diverse pathologies, including male reproductive health where selenium deficiency downregulates these proteins and impairs cell viability [30]. In the OA context, the observed lack of significant serum biomarker differences between patients and controls may reflect systemic compensatory mechanisms or heterogeneity in disease phenotypes, paralleling studies that identified distinct OA phenotypes with variable progression risks [34]. The nuanced interplay between demographic factors and ferroptosis-related protein dynamics warrants further mechanistic dissection, particularly to elucidate how these variables modulate local joint microenvironmental responses and influence biomarker utility.

Finally, the integration of SLC7A11 and GPX4 measurements with established radiographic grading systems and patient-reported outcomes proposes a multi-dimensional approach to OA assessment. This complements recent advances in imaging-based prognostication, including MRI radiomics that enhance risk stratification beyond conventional Kellgren-Lawrence grading [35], and machine learning models incorporating biochemical and clinical variables to predict structural progression [36]. By incorporating ferroptosis biomarkers, our approach enriches the predictive framework with molecular insights, potentially enabling earlier identification of high-risk patients and monitoring of therapeutic efficacy. This methodological innovation advances OA research towards precision medicine paradigms that integrate molecular, imaging, and clinical data for comprehensive disease management.

The limitations of this study are multifaceted. Firstly, while we utilized a substantial sample size, the absence of longitudinal follow-up data restricts our ability to draw definitive causal inferences regarding the relationship between SLC7A11 and GPX4 levels and the progression of knee osteoarthritis (OA). This limitation is compounded by the potential homogeneity of our patient population, which may not adequately represent the diverse characteristics observed in broader OA cohorts. Secondly, the reliance on ELISA for biomarker quantification, although a widely accepted method, introduces potential variability due to inter-assay differences and may not capture the dynamic changes in biomarker levels over time or in response to specific treatments, thus limiting the reproducibility of our findings across different clinical settings. Thirdly, this study was limited to the analysis of only GPX4 and SLC7A11 concentrations. Additional studies examining change of

other ferroptosis related markers may potentially yield more impactful results. Since our main aim was to observe the before- and after-treatment level changes of GPX4 and SLC7A11, and to also observe whether the changes of GPX4 and SLC7A11 levels are associated with the promotion of clinical symptoms, the treatment arm lacks any control group, blinding, and randomization.

In conclusion, this study suggests that SLC7A11 and GPX4 concentrations in synovial fluid may serve as promising biomarkers for assessing disease severity and treatment responses in knee OA. The observed negative correlation with established clinical and radiographic measures underscores their potential utility in clinical practice. Future research should focus on validating these findings in larger and more diverse populations, exploring the mechanistic pathways of these biomarkers and assessing their applicability in real-world clinical settings to enhance patient management and therapeutic strategies.

Declaration of Generative AI in Scientific Writing

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Declaration of Interest:

The authors declare that they have no known competing financial interests.

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