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Original Article

Association of follistatin-like 3 concentrations in serum and synovial fluid with the radiographic severity of knee osteoarthritis

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Abstract: Objective: Follistatin-like 3 (FSTL3), a circulating glycoprotein, is correlated with obesity and inflammation, which are potential mechanisms of osteoarthritis (OA). This study aims to determine the correlation of FSTL3 concentrations in serum and synovial fluid (SF) with the radiographic severity of OA. Methods: This study consisted of 200 patients with knee OA and 148 healthy controls. The radiological grading of OA in the knee was performed in accordance with Kellgren-Lawrence (KL) grading system. Results: Knee OA patients had higher serum FSTL3 concentrations compared with healthy controls. Knee OA patients with KL grade 4 showed significantly elevated FSTL3 concentrations in serum and SF compared with those with KL grades 2 and 3. Moreover, knee OA patients with KL grade 3 had significantly higher FSTL3 concentrations in serum and SF compared with those with KL grade 2. FSTL3 concentrations in serum and SF of knee OA patients were significantly correlated with KL grading criteria. Conclusions: FSTL3 concentrations in serum and SF are correlated with the radiographic severity of OA.

Keywords: Follistatin-like 3, serum, synovial fluid, severity, osteoarthritis

Introduction

Osteoarthritis (OA), a chronic degenerative joint disease, is characterized by articular cartilage degradation, bony outgrowth at joint margin, osteophyte formation, subchondral sclerosis, and synovitis [1]. OA causes pain, stiffness, reduced motion, swelling, crepitus, and disability, thereby leading to huge health costs worldwide [2]. Some factors such as aging, overweight, female gender, smoking, genetics, and joint injury are considered to be risk factors for OA [3]. In addition, inflammation also contributes to the pathogenesis of OA. Various evidences indicated the presence of intra-articular low-grade inflammation during the development and progress of OA [4].

Follistatin-like 3 (FSTL3), a secreted glycoprotein, is secreted by the reproductive system, adipose tissues, pancreas, liver, and skeletal muscles [5]. FSTL3 formes high-affinity complexes with transforming growth factor-β (TGF-β) family members, most notably activin A and myostatin and thereby inhibiting their function [6]. FSTL3 plays a key role in regulating inflammation. The promoter region of FSTL3 gene contains an NF-κB binding site, and FSTL3 protein expression is elevated in response to tumor necrosis factor-α (TNF-α) stimulation in HepG2 cells [7]. Given that inflammation is an important mechanism in the development and progression of OA, we hypothesized that FSTL3 may be involved in the pathogenesis of OA.

This study is performed to determine the correlation of FSTL3 in serum and synovial fluid (SF) with the presence and radiographic severity of OA.

Materials and methods

Patients

A total of 200 patients diagnosed with knee OA in accordance with the criteria of the American College of Rheumatology were enrolled in this study. Patients with infectious or inflammatory arthritis, knee injury, aseptic osteonecrosis, and congenital abnormality, who are receiving

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

Characteristics	Knee OA patients (n=200)	Healthy controls (n=148)	*P* value
Age (years)	60.76±10.34	59.96±9.21	0.455
Gender (male/female)	77/123	52/96	0.521
FSTL3 in serum (ng/mL)	23.76 (19.38-27.40)	19.18 (14.98-23.55)	<0.001
FSTL3 in SF (ng/mL)	8.00 (6.74-9.72)		

various drugs such as analgesics and non-steroidal anti-inflammatory drugs, were excluded from this study. Control participants of 148 subjects were recruited and matched to the cases in teams of age and gender. These controls presented neither clinical nor radiological evidence of OA, arthritis, or other joint diseases.

This study was approved by the research ethics committee of our hospital. Written informed consent was obtained from all patients and healthy volunteers prior to their participation in the study.

Radiographic assessment of OA

Disease severity was graded using the system of Kellgren-Lawrence (KL) system. OA patients were defined as having radiographic knee OA of KL grade ≥2 in at least one knee. Healthy controls were defined as having no radiographic knee OA as indicated by KL grades of 0 for both knees. The higher grade of the two knees was used for analysis.

Laboratory methods

Venous blood was obtained in the fasting state at 7:00 am after overnight. Before any treatment on OA, SF was obtained from OA patients who received the treatment of hyaluronic acid injection for the first time. The FSTL3 concentrations in serum and SF were quantified using enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN, USA).

Statistical analysis

Data are presented as means ± SD or median (interquartile range). Characteristics of knee OA patients and healthy controls were compared using Unpaired t-test, Mann-Whitney U test, or Chi-square test. Kruskal-Wallis test was utilized to compare the differences of FSTL3 concentrations in serum and SF among knee OA patients with different KL grades. The correlation of FSTL3 concentrations in serum and SF with disease severity was determined by Spearman correlation analysis and a multinomial logistic regression analysis. All statistical analysiss were performed using SPSS for window 13.0. Differences were considered significant at $P<0.05$.

Results

Baseline clinical parameters

No significant differences were found in age and gender between patients with knee OA and healthy control (**Table 1**).

The FSTL3 concentrations in serum and SF

Patients with knee OA had significantly higher serum FSTL3 concentrations compared with healthy controls ($P<0.001$) (**Table 1**).

FSTL3 concentrations in knee OA patients with different KL grades

The FSTL3 concentrations in serum and SF of knee OA patients with different KL grades are shown in **Table 2**. Knee OA patients with KL grade 4 had significantly elevated FSTL3 concentrations in serum and SF than those with KL grade 2 and 3. Furthermore, the FSTL3 concentrations in serum and SF of knee OA patients with KL grade 3 were significantly higher compared with those with KL grade 2.

Association of clinical parameters with KL grades

As shown in **Figures 1** and **2**, the FSTL3 concentrations in serum and SF were associated with KL grades ($r=0.344$, $P<0.001$ and $r=0.414$, $P<0.001$ respectively) by Spearman correlation analysis. Multinomial logistic regression analysis showed that FSTL3 concentrations in serum and SF were both positively correlated with KL grades ($P<0.001$ and $P<0.001$ respectively).

Discussion

In this study, we found that FSTL3 concentrations in serum were elevated in knee OA

Table 2. The FSTL3 concentrations of serum and SF in knee OA patients with different KL grades

FSTL3 (ng/mL)	Grade 2 (n=62)	Grade 3 (n=89)	Grade 4 (n=49)	*P* value
Serum	21.17 (17.12-24.88)[b]	23.83 (19.66-27.12)[a]	26.33 (22.37-28.70)[a,b]	<0.001
SF	7.23 (6.25-8.20)[b]	8.11 (6.76-9.40)[a]	9.66 (7.49-10.62)[a,b]	<0.001

[a]*P*<0.01 vs KL grade 2; [b]*P*<0.01 vs KL grade 3.

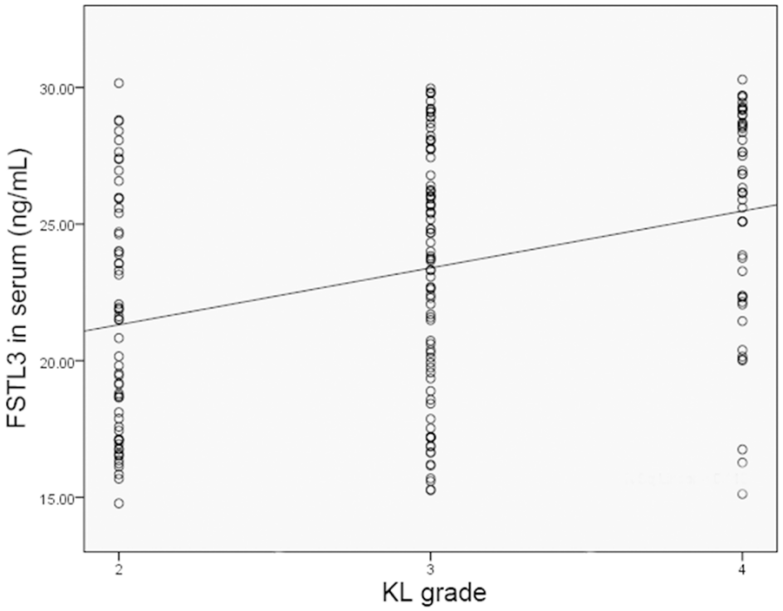

Figure 1. Correlation of serum CCL18 levels with different KL grades.

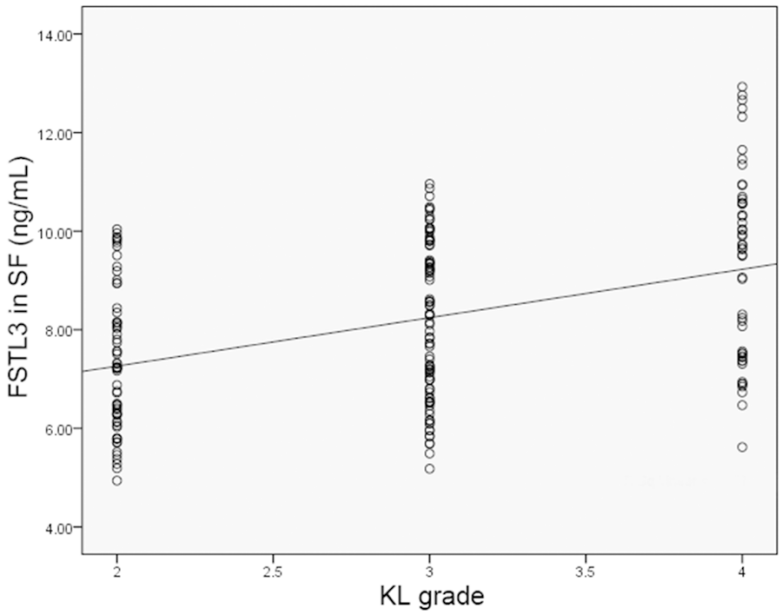

Figure 2. Correlation of SF CCL18 levels with different KL grades.

patients compared with healthy controls. FSTL3 concentrations in serum and SF of knee OA patients were correlated with the disease severity, as evaluated using the KL grading criteria. To the best of our knowledge, this study is the first to demonstrate the association of FSTL3 concentrations in serum and SF with the radiographic severity of knee OA.

At present, OA severity is mainly assessed by radiological grading with magnetic resonance imaging and direct arthroscopic examination. However, these methods are limited because of high costs, dispute regarding critical standards, and traumatic defects [8]. Biomarkers are currently utilized in early diagnosis of different diseases [9]. The present study revealed that FSTL3 concentrations in serum and SF were closely related with the radiographic severity of OA. This result indicates that serum and SF FSTL3 concentrations can serve as a novel biomarker for grading the progression of OA.

Obesity is recognized as the strongest modifiable risk factor in OA. However, the exact mechanism of obesity resulting in OA remains unclear. Excessive joint loading as a result of increased body weight was previously considered to be the main pathogenesis of OA [10]. Inflamed adipose tissues and dyslipidaemia were recently shown to play pivotal roles in obesity-induced OA [11]. Moreover, FSTL3 is closely correlated with obesity development. Allen et al reported that FSTL3 mRNA expression levels were significantly increased in subcutaneous

fat of ob/ob mice compared with wild-type mice [5]. Furthermore, injection of recombinant leptin to ob/ob mice decreased the FSTL3 mRNA levels in both subcutaneous and visceral fats [5]. FSTL3 knockout mice exhibited reduced perigonadal fat pad weights compared with the controls, indicating that FSTL3 plays a role in regulating of body composition [12]. In addition, plasma FSTL3 concentrations were increased in obese subjects compared with lean ones independent of glycemic state [13]. Plasma FSTL3 concentrations were positively correlated with fat mass and fasting insulin [13]. Overall, these results indicate the key role of FSTL3 in the development of obesity, leading to the possible conclusion that FSTL3 is involved in the link of obesity and OA development.

Inflammation is involved in the mechanism of OA. Inflammatory cytokines in the OA joint have been shown to play an important role in maintaining the catabolic processes of chondrocytes involved in OA disease progression [14]. OA synovial fibroblasts have been implicated in sustaining the damage found in the OA joint by secreting proinflammatory cytokines [15]. FSTL3 was recently found to be associated with inflammation. Plasma FSTL3 concentrations were positively correlated with plasma TNF-α and interleukin-6 (IL-6) concentrations [13]. Furthermore, infusion of lipopolysaccharide and TNF-α increased plasma FSTL3 concentrations in humans [13]. Therefore, FSTL3 may partly contribute to the development and progression of OA through inflammation-induced effects.

The limitations of this present study should also be considered. First, this cross-sectional study is performed in a relatively small number of samples. Therefore, the findings should be validated by further longitudinal studies in a larger population. Second, we did not assess FSTL3 concentrations in SF from healthy controls because of ethical concerns.

In conclusion, FSTL3 concentrations in serum and SF were positively correlated with the severity of knee OA. FSTL3 concentrations in serum and SF may serve as a novel biomarker in addition to the traditional methods for assessing the risk and severity of knee OA.

Disclosure of conflict of interest

None.

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