Increased Serum AXL Is Associated With Knee Osteoarthritis Severity

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Research Article

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Abstract

Objective: Serum AXL expression and clinical significance in patients with knee osteoarthritis (KOA).

Methods: 183 patients with knee osteoarthritis were selected and divided based on the Kellgren-Lawrence (KL) score into KL 0 subgroups (n = 42), KL I-II subgroups (n = 90), and KL III-IV subgroups (n = 51). Healthy volunteers (n= 170) in our hospital were selected as the control group. AXL level in serum was detected by enzyme-linked immunosorbent assay (ELISA). The correlation between serum AXL with severity and clinical indicators of osteoarthritis was analyzed.

Results: The level of serum AXL was significantly higher in the osteoarthritis group than that in the control group ($P<0.05$). In the osteoarthritis patients, serum AXL level was increased with the increase of KL score. Serum AXL level was positively correlated with age, body mass index (BMI), erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP), cartilage oligomeric protein (COMP), matrix metalloproteinase-13 (MMP-13), and transforming growth factor-β1 (TGF-β1) levels. The cut-off value for serum AXL was determined as 33.375 ng/mL by receiver operating curve (ROC) analysis.

Conclusion: The level of serum AXL in patients with osteoarthritis is abnormally high, which is closely related to the severity of osteoarthritis.

Introduction

Osteoarthritis (OA) is a degenerative disease, and it primarily affects hips and knees as major weight-bearing joints [1]. Over the past two decades, knee osteoarthritis (KOA) has become fastest basis of expanding physical disability with a significant economic burden worldwide [2, 3]. KOA is the most common form of arthritis, and characterized by joint degeneration, loss of cartilage, osteophyte formation, cysts and alterations of subchondral bone [4, 5]. Besides that, a broad range of mechanical and biochemical inflammatory mediators (pro-inflammatory cytokines, growth factors and matrix metalloproteinase) contribute to the pathogenesis of KOA [6, 7]. Till to date, the prime cause of KOA development is still unidentified and optimal treatment remain elusive. Studies shows that more than 40 million Americans having OA, and 80% among them older than 50 years [8]. Other studies shows that knee osteoarthritis prevalence rate in Chinese and Japanese population is also cumulative. The prevalence rate in in Chinese population reached 15.6% in over 40 years aged groups [9]. Beside that the prevalence rate of KOA in Japanese population reported as up to 42.0 % in men and 62.4 % in women over 40 aged groups [10]. Therefore, knee osteoarthritis (KOA) is a global issue in elderly population and early diagnosis is required to begin possible treatment.

Radiography imaging technique is viewed as a gold standard method for diagnosis of KOA, but the current imaging technique is suffering with the sensitivity and specificity [11]. Although, imaging technique allows detection OA of the knee: joint space narrowing, presence of osteophytes, subchondral sclerosis, and cysts. Due to lack of sensitivity and specificity of radiographic imaging technique, there is an urgency to develop a potentially alternative tool for diagnosis of KOA. Body fluid serum routinely

tested in clinics for diagnosis, and treatment of different diseases. They are very decisive medium and harbor plenty of biomarker for the monitoring of our health. Earlier basic and clinical studies revealed several biomarkers are known to be correlated with the extent of OA on radiography of the knee and being proposed as diagnostic tools [12–15]. However, the currently used biomarkers are inadequate for prognosis of OA.

The receptor tyrosine kinase AXL is a 140-kDa protein that belongs to a tyrosine kinase receptor (TAM) subfamily, together with Tyro3 and Mer. The TAM receptors (AXL, Tyro3 and Mer) play critical role in innate immune homeostasis and vitamin-K dependent ligand growth arrest specific protein 6 (GAS6) can binds all three receptors with highest affinity for AXL [16, 17]. Transmembrane protein AXL can be cleaved proteolytically at its extracellular membrane domain (EMD) and subsequently released as soluble AXL, which can be detected in serum or plasma [18, 19]. Furthermore, studies revealed that targeted delivery of TAM receptor ligand genes Gas6 diminishes the arthritis pathology effectively but endogenous role of AXL in arthritis development is not fully understood [20]. We hypothesized that AXL concentration is correlated with the severity of KOA and can predict the development and progression of KOA as seen on radiography of the knee.

In the present study, we analyzed AXL levels in sera from participants suffering from KOA and divided in different groups according to the Kellgren-Lawrence (KL) score. We assess the diagnostic performance of AXL for KOA in comparison to participant control groups. Furthermore, we were able to determine the accuracy of AXL in KOA in different groups that demonstrating the potential diagnostic value of AXL for routine clinical use in surveillance of patients at high risk for KOA severity.

Materials And Methods

Subjects

This study included 183 patients with knee osteoarthritis (KOA) who admitted to our hospital from July 2019 to December 2020. There were 102 males and 81 females, with a median age of 68 years old. The patient's inclusion criteria were as follows: (1) All patients were diagnosed as knee osteoarthritis with duration of disease > 6 months; (2) All patients had radiological evidence of osteoarthritis with a Kellgren-Lawrence (KL) score of 0-IV. All patients were graded as KL according to the X-ray pictures of bone and joint: grade 0: no change; grade I: slight osteophyte; grade II: obvious osteophyte, no joint space involved; grade III: moderate narrowing of joint space; grade IV: joint space narrowing, subchondral osteosclerosis [21]. There were 42 cases of grade 0, 51 cases of grade I, 39 cases of grade II, 28 cases of grade III and 23 cases of grade IV in 183 patients. The patient's exclusion criteria are as follows: (1) Patients had concurrent systemic or local inflammation, infection, trauma, tumor, connective tissue disease, and autoimmune disease; (2) Patients received any intra-articular injections within months or systemic glucocorticoids within 3 months; (3) Patients had history of knee injury and operation, rheumatoid arthritis, ankylosing spondylitis, and severe osteoporosis. The serum samples of 170 age and sex matched healthy people in our hospital during the same period were selected as the control group,

including 91 males and 79 females, with an median age of 64 years old. The age, gender, height, and weight of all participates were collected. This study was approved by Institutional Review Board (IRB) of the hospital ethics committee. All subjects provided written informed consents before sample collection. All experimental protocols were approved by the Ethics Committee of the hospital.

Specimen collection and preparation

Whole blood was collected from fasting participants in the morning. Blood samples were centrifuged (15000×g for 10 min at 4°C) to separate serum. The processed serum supernatant was then aliquoted into a 1.5 mL Eppendorf tube and stored at -80°C for further analysis.

Measurement of serum proteins and cytokines concentration

The serum concentrations of cytokines and proteins were determined using enzyme-linked immunosorbent assay kits, including CRP (DCRP00, R&D Systems), COMP (DCMP0), MMP-13 (DY511), TGF-β1 (DY240), Gas6 (DY885B), and AXL (DAXL00), according to the manufacturer's protocol. In brief, serum sample was directly transferred or diluted to the wells of the ELISA plate, and the absorbance was measured in a microplate reader. The protein levels were quantified by their corresponding standard curve.

Statistical analysis

SPSS 20.0 software was used for statistical analysis. The measurement data were expressed as median (interquartile range), and analyzed by one-way ANOVA or Manne-Whitney U test. The correlation between serum AXL level and other clinical indicators was analyzed by Spearman correlation. Receiver operating curve (ROC) analysis was carried out to determine the diagnostic potential of AXL for KOA, the difference was statistically significant ($P < 0.05$).

Results

Baseline demographic and clinical characteristics of subjects

We enrolled a total of 183 OA patients in this study. The baseline demographic and clinical characteristics are shown in Table 1. There was no significant difference in age and gender between control group and OA group. The mean BMI and ESR were significantly higher in OA patients with respect to healthy controls (both $P < 0.05$). Analysis of biochemical parameters revealed significantly higher serum CRP, COMP, MMP-13, TGF-β1 and Gas6 levels in the OA group compared to control group (all $P < 0.05$).

Table 1
Demographic and clinical characteristics of osteoarthritis patients and healthy controls

Clinical parameters	Control (n = 170)	Osteoarthritis (n = 183)	P value
Age	66.0 (59.8–72.0)	68.0 (61.0–74.0)	0.223
Gender (male)	91 (53.5%)	102 (55.7%)	0.677
BMI (kg/m2)	23.8 (21.4–25.5)	25.0 (23.4–26.4)	< 0.001
ESR (mm/h)	8.0 (6.8–9.3)	18.1 (16.9–20.2)	< 0.001
CRP (μg/mL)	3.4 (3.2–3.6)	6.0 (5.6–6.6)	< 0.001
COMP (ng/mL)	14.4 (13.3–15.6)	25.8 (23.8–28.7)	< 0.001
MMP-13 (ng/mL)	12.5 (11.5–13.9)	21.8 (19.0-23.7)	< 0.001
TGF-β1 (pg/mL)	12.5 (11.1–13.8)	16.6 (15.2–18.8)	< 0.001
Gas6 (ng/mL)	16.6 (15.0-18.3)	24.6 (22.1–26.9)	< 0.001

BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; COMP, cartilage oligomeric protein; MMP-13, matrix metalloproteinase-13; TGF-β1, transforming growth factor-β1; Gas6, growth arrest-specific gene 6 protein. Mann-Whitney test or $\chi 2$ test was performed.

Serum AXL concentrations were elevated in OA patients

We analyzed the concentration of serum AXL in the OA patients and healthy controls by non-parametric tests. The serum AXL level was significantly higher in the OA patients [43.45 (36.61–50.33) ng/mL] compared with that in the healthy control [27.38 (24.25–29.82) pg/mL] ($P < 0.001$; Fig. 1A). Moreover, among OA patients, serum AXL level was significantly higher in subjects with KL I-II grade compared to subjects with KL 0 grade, higher in subjects with KL III-IV compared to subjects with KL I-II grade (both $P < 0.001$; Fig. 1B).

Correlation between serum AXL level and clinical indicators

Spearman correlation test was used to determine the relationship between serum AXL and other biochemical indexes. In OA patients, serum AXL was significantly positively correlated with BMI, ESR, CRP, COMP, MMP-13 and TGF-β (Table 2). There was no significant correlation between serum AXL and Gas6 in the control group ($r = 0.105$, $P = 0.175$; Fig. 2A). However, in the OA group, serum AXL showed significant positive correlation with Gas6 ($r = 0.327$, $P < 0.001$) (Fig. 2B).

Table 2
Correlation between serum AXL and clinical indicators

	r	P
Age	0.058	0.437
BMI (kg/m2)	0.149	0.044
ESR (mm/h)	0.210	0.004
CRP (μg/mL)	0.221	0.003
COMP (ng/mL)	0.190	0.010
MMP-13 (ng/mL)	0.287	< 0.001
TGF-β1 (pg/mL)	0.192	0.009
Spearman correlation was performed.		

Diagnostic potential of AXL for knee osteoarthritis

Then the receiver operating characteristic (ROC) curve was drawn. The optimal cut-off value of serum AXL level in the diagnosis of osteoarthritis was 33.375 ng/mL, with 85.8% sensitivity and 92.9% specificity (AUC = 0. 951, 95%CI = 0.929–0.973; P < 0.001) (Fig. 3).

Discussion

Clinical findings are more important than radiographs in OA knees to decide further course of management [22–24]. Radiographs alone do not reflect the clinical severity of knee OA and routine use of radiographs in management of knee osteoarthritis is not recommended [23]. In this study we determine the clinical value of serum levels AXL with other biochemical molecules (CRP, COMP, MMP-13, TGF-β and GAS6) for diagnosis and assessment of disease severity of knee osteoarthritis. The baseline analysis and biochemical parameters revealed that OA patients have significant higher concentration of CRP, COMP, MMP-13, TGF-β1 and Gas6 compared to control group (Table 1). These findings are inline of several other studies indicate that these biochemical parameters involved in osteoarthritis development and treatments [25–29]. In addition, we observed that serum AXL level in OA patients was significantly higher compared with that in the healthy control (Fig. 1A). Thus, AXL have the ability to distinguish OA patients from healthy control. Furthermore, spearman correlation analysis revealed that serum AXL level is positively correlated with clinical biochemical indicators BMI, ESR, CRP, COMP, MMP-13 and TGF-β (Table 2). Moreover, a significant positive correlation was observed between serum level AXL and Gas6 in OA patients' groups and no correlation among control groups (Fig. 2). Besides that, serum AXL shown a diagnostic accuracy with an AUC of 0.951 (cut-off 33.37 ng/ml) for the detection of osteoarthritis with

85.8% sensitivity and 92.9% specificity (AUC = 0. 951, 95%CI = 0.929–0.973; P < 0.001) compared to healthy controls.

Current study revealed positive correlations among serum AXL levels and the radiographic severity of knee OA, and Kellgren-Lawrence (KL) score. AXL expression increased with an increasing radiographic disease severity (KL grade). The AXL level was significantly higher in subjects with KL I-II grade compared to subjects with KL 0 grade, higher in subjects with KL III-IV compared to subjects with KL I-II grade. Thus, based on our biochemical analysis, we revealed that AXL cutoff value could be useful during screening for abnormal knee osteoarthritis (KOA), when used in combination with conventional imaging tools for severity assessments.

The perspective of this study is current bottlenecks in KOA drug development and contribute to patient's stratifications for more efficacious treatment of KOA. Serum AXL level might be an indicator to facilitate better clinical decision-making concerning treatment of KOA patients and severity assessments.

Conclusion

In this study we found that serum AXL level were significantly elevated in KOA patients and we identified a positive correlation between serum AXL levels with the degree severity in KOA patients. Measurement of AXL level in the serum can be used as an alternative biomarker to assess the progression of OA in addition to the use of traditional methods for assessing the risk and severity of KOA. The diagnostic accuracy of AXL in KOA diseases should be confirmed in different patient cohorts.

Declarations

Acknowledgment

none

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Our study has been approved by the medical ethics committee of the Shanghai Kaiyuan Orthopedic Hospital and written informed consent were obtained from all included patient's.

Consent for publication

Not applicable

Competing interests

The authors declare no competing financial interests.

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Figures

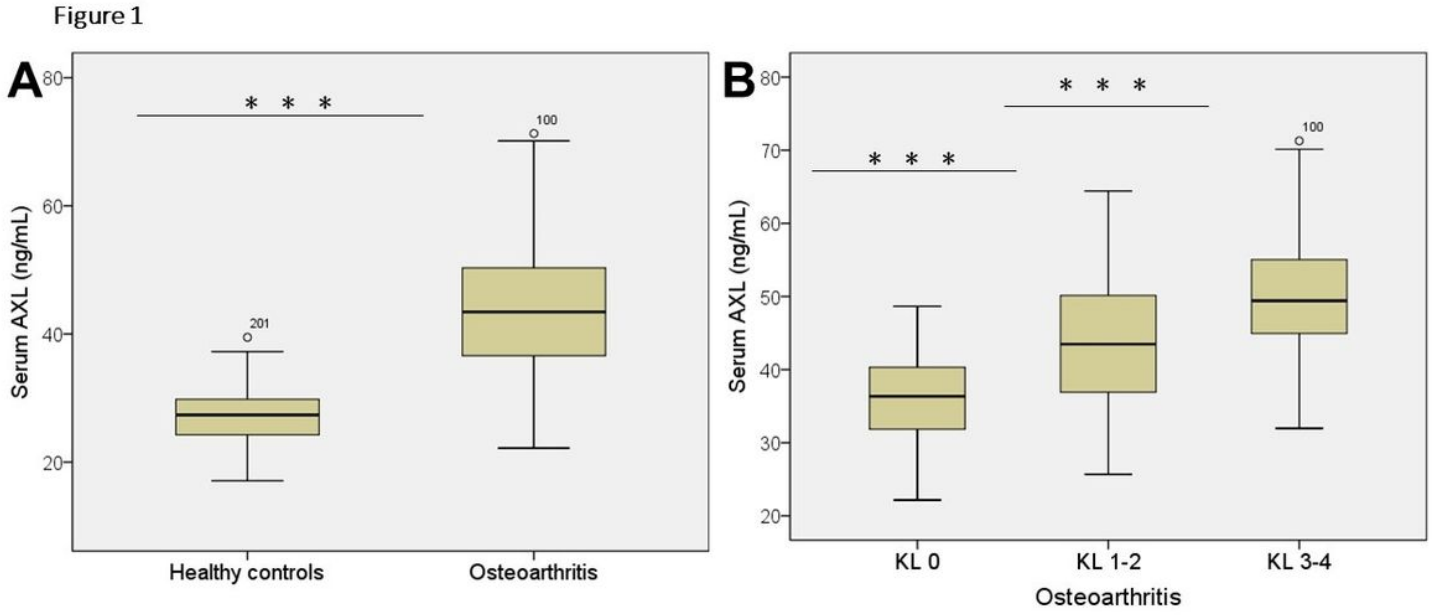

Figure 1

Serum AXL levels in OA patients. (A) Comparison of serum AXL between patients with knee OA (KLI-III) and healthy subjects; (B) Comparison of serum AXL between KL 0, KL I-II, and KL III-IV in patients with knee OA. ***P<0.001.

Figure 2

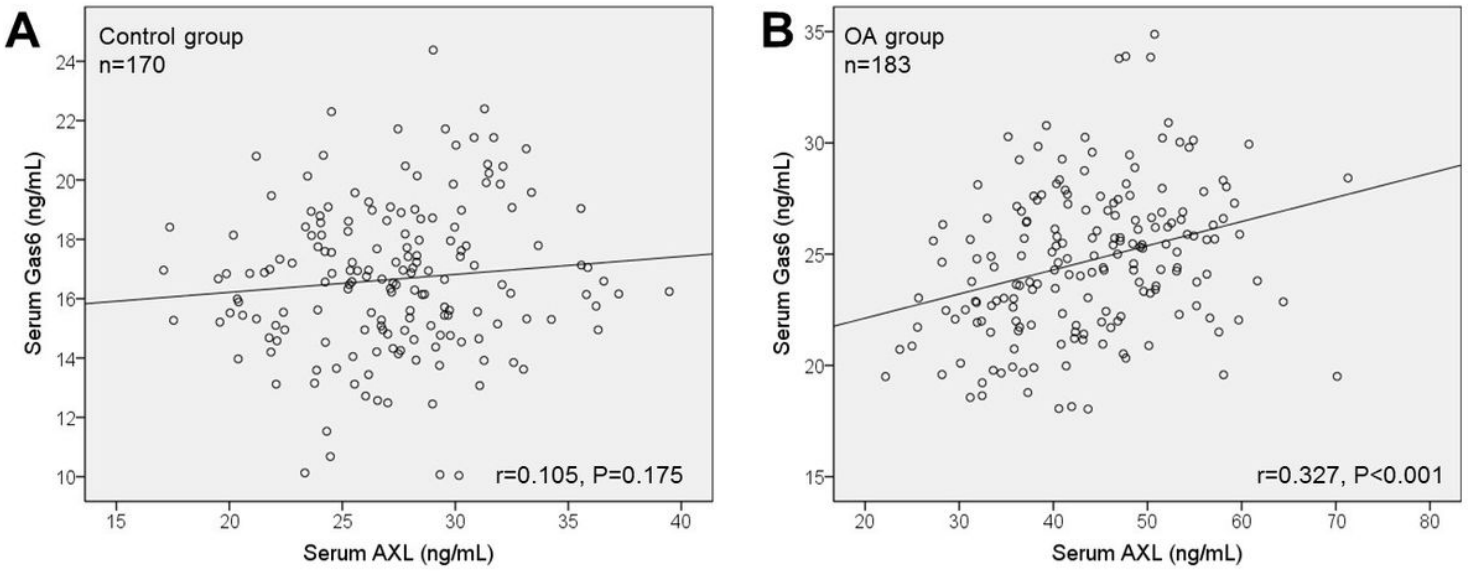

Figure 2

The correlation between serum AXL and clinical parameters in OA patients. Spearman correlation test was performed. ***P<0.001.

Figure 3

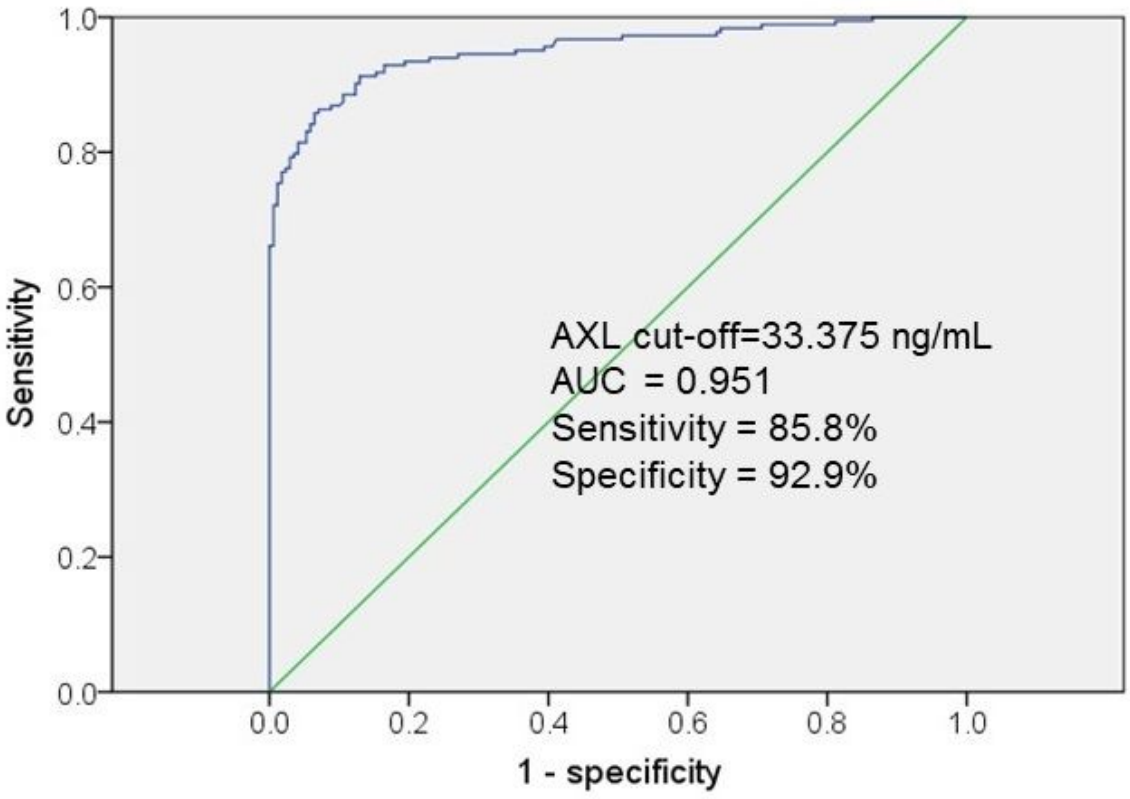

Figure 3

Receiver operating curve analysis of serum AXL levels discriminating between patients with knee OA and healthy subjects.