Serum Leukocyte Cell-derived Chemotaxin 2 (LECT2) Level is Associated with Osteoporosis

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Research Article

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Abstract

Background

Previous studies have shown that leukocyte cell-derived chemotaxin 2 (LECT2), a hepatokine, is associated with obesity and non-alcoholic fatty liver disease (NAFLD). It is well known that hepatokines play important roles in mediating interactions among bone, adipose tissue, and liver. We sought to examine serum LECT2 levels in subjects with osteoporosis (OP) to confirm its association with OP.

Methods

From March 2019 to March 2020, a total of 96 adult subjects (52 OP patients and 44 controls) visiting the 2nd Spine Department of the Affiliated Hospital of School of Medicine of Ningbo University were recruited. The bone mineral density (BMD) of all subjects were assessed by dual-energy X-ray (DXA). Blood samples were collected for measurements of high-sensitivity C-reactive protein (hs-CRP), plasma glucose (PG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglycerides (TG), creatinine and uric acid. Serum LECT2 levels of total 96 participants were measured by enzyme linked immunosorbent assay (ELISA). The relationships between serum LECT2 levels and biomedical parameters were analyzed using the Spearman correlation coefficient.

Results

Serum LECT2 levels in OP patients were significantly higher than that of healthy controls (29.57 ng/mL VS 19.82 ng/mL, $P < 0.01$). To confirm the role LECT2 played in OP, we found a significantly negative correlation in all subjects between serum levels of LECT2 and lumbar BMD, as well as femoralneck BMD. A significantly positive correlation in all was observed between serum levels of LECT2 and TC, whereas there was a significantly negative correlation between serum levels of LECT2 and creatinine. Meanwhile, serum LECT2 levels were measured to diagnose OP patient by plotting receiver-operating characteristic (ROC) curve, the area under the ROC curve was 0.729 ($P < 0.01$). The optimal cutoff point for LECT2 concentration to diagnose OP patient was 16.44 ng/mL.

Conclusions

We showed that serum LECT2 levels were significantly up-regulated in OP patients, and LECT2 levels were significant positively associated with total cholesterol and negatively associated with creatinine. It could be a potential biomarker for OP diagnosis.

Background

Osteoporosis (OP) is a highly prevalent systemic bone metabolic disease, which caused by bone microstructure destruction [1, 2]. It is characterized by bone mass reduction, leading to increased bone fragility and fracture risk. OP negatively affects the quality of life and leads to fracture and even death. It has become a global problem affecting the health of aging population [3, 4]. OP occurs when the balance

between bone resorption and formation broken. Previous studies have showed that immune system played a pivotal role in bone homeostasis [5]. For example, inflammatory cytokines, interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF) and macrophage colony-stimulating factor (M-CSF), tightly regulate osteoclastogenesis and bone resorption [6–9], and their imbalance could lead to OP.

Leukocyte cell-derived chemotaxin 2 (LECT2), a 16 kDa chemotactic protein with multiple physiological functions, is mainly expressed in human hepatocytes and secreted into the blood [10]. Early studies have reported that LECT2 acts as a regulator of immune and inflammatory response and plays a major role in various pathophysiological processes, such as sepsis [11], hepatitis [12], arthritis [13], and hepatocarcinoma [14]. LECT2 also implicated in metabolic disorder diseases, including obesity [15], diabetes [16], and non-alcoholic fatty liver disease (NAFLD) [17].

Recently, studies showed that hepatokines were involved in the complex interactions among bone, adipose tissue, and liver [18–22]. Additionally, it has been reported that LECT2 modulates TNF expression via the CD209a receptor, which is on the surface of osteoclasts and macrophages [23]. As a multifunctional protein mainly secreted by hepatocytes and closely associated with obesity and NAFLD, we hypothesize that LECT2 might be also involved in the process of OP, while the clinical significance of serum LECT2 in OP patients is unavailable. In the present study, we evaluated the levels of serum LECT2, high-sensitivity C-reactive protein (hs-CRP), plasma glucose (PG), creatinine, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglycerides (TG) in 52 OP patients and 44 healthy subjects. The relationships among LECT2, osteoporosis, and metabolic parameters were investigated.

Material And Methods

Subjects

From March 2019 to March 2020, a total of 96 adult subjects visiting the 2nd Spine Department of the Affiliated Hospital of School of Medicine of Ningbo University were recruited. Subjects with malignant tumor, renal, liver, and respiratory dysfunction, liver cirrhosis, infection, inflammatory rheumatism, and in use of steroids or other drugs that could cause osteoporosis were excluded.

Patients affected by OP (lumbar spine and/or femoral neck T score ≤ -2.5) and patients with either osteopenia or normal controls were recruited consecutively. The bone mineral density (BMD) of all subjects were assessed by dual-energy X-ray (DXA, Discovery Wii, Hologic) at the spine (L1-L4) and at the hip (femoral neck). After admission, blood samples were collected after an overnight fasting for measurements of hs-CRP, PG, ALT, AST, TC, TG, creatinine and uric acid. All clinical and metabolic data collected from both osteoporosis group and control group are shown in Table 1.

Table 1
Clinical and metabolic data of osteoporosis patients and healthy controls.

	Osteoporosis(n=52)	Control(n=44)	<i>P</i>
Age(year)	66.65±7.92	64.32±8.50	0.167
Female(n)	40	20	
Lumbar BMD (T-score) *	-3.09±0.94	-0.38±1.36	0.000
Femoralneck BMD (T-score) *	-2.12±0.98	-0.63±0.94	0.000
Hs-CRP (mg/L)	1.54±0.18	1.84±0.28	0.139
ALT (U/L)	18.32±14.50	23.20±19.91	0.169
AST (U/L)	21.75±6.66	23.45±10.82	0.347
PG (mmol/L)	5.50±0.93	5.64±1.26	0.527
TC (mmol/L)	4.48±1.03	4.57±0.98	0.634
TG (mmol/L) *	1.19±0.55	1.56±0.71	0.005
Creatinine (μmol/L) *	58.11±15.13	68.19±13.49	0.001
Uric acid (μmol/L) *	292.53±88.43	341.02±101.65	0.014
Abbreviation: BMD: bone mineral density; hs-CRP: High-sensitivity C-reactive protein; ALT: alanine aminotransferase; AST: aspartate amino transferase; PG: plasma glucose; TC: total cholesterol; TG: triglycerides.			

Blood samples were collected and stored at -80°C immediately for both in OP patients and healthy controls at admission. Subsequently, serum LECT2 levels were measured by a commercially available human LECT2 ELISA kit (R&D system, Minneapolis, USA) according to the manufacture's protocol.

Written informed consents were obtained from all participants and all experiments were approved by the institutional review board of the Affiliated Hospital of School of Medicine of Ningbo University.

Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics Version 22.0 (IBM, New York, USA). The Student's t-test was used to analyze continuous variables. A two-tailed unpaired student's t-test was used to compare the serum LECT2 levels between OP and control subjects. The relationships between serum LECT2 levels and biomedical parameters were analyzed using the Spearman correlation coefficient. Receiver-operating characteristic (ROC) curve was used to evaluate the accuracy of LECT2 to diagnose OP. *P* value less than 0.05 was considered statistically significant.

Results

Serum LECT2 levels were significantly increased in OP patients than that of controls (29.57 ± 15.88 ng/mL VS 19.82 ± 10.75 ng/mL, $P < 0.01$; Fig. 1), whereas creatinine, uric acid serum levels were significantly lower in OP patients than that of controls.

There was a significantly negative correlation in all subjects between serum levels of LECT2 and lumbar BMD ($r = -0.347$, $P = 0.001$; Fig. 2), as well as between serum levels of LECT2 and femoralneck BMD ($r = -0.219$, $P = 0.033$; Fig. 3). A significantly positive correlation in all was observed between serum levels of LECT2 and TC ($r = 0.269$, $P = 0.008$; Fig. 4), whereas there was a significantly negative correlation between serum levels of LECT2 and creatinine ($r = -0.205$, $P = 0.045$; Fig. 5).

As revealed by the ROC curve analysis, serum LECT2 levels were performed to detect OP patients, the area under the ROC curve was 0.729 ($P < 0.001$). The optimal cutoff point of LECT2 concentration to diagnose OP patient was 16.44 ng/mL. By this cutoff value, diagnostic efficiency for OP reached the highest value with sensitivity and specificity of 98.1% and 47.7% , respectively (Fig. 6).

Discussion

LECT2 is a hepatokine mainly expressed in hepatocytes and endothelial cells [24]. It has been reported as an immune modulatory factor in inflammatory arthritis [25], bacterial sepsis, renal amyloidosis [11, 26, 27], hepatitis, and hepatic carcinogenesis. Yoo et al. [17] reported an increase in plasma LECT2 of NAFLD group compared with control subjects (31.2 ng/mL VS 24.5 ng/mL). Tanisawa et al. [28] showed that participants with dyslipidemia had higher levels of plasma LECT2 than those without dyslipidemia, which consistent with the different LECT2 levels between participants with or without metabolic syndrome.

Some metabolic syndromes, such as obesity and NAFLD, were described to be associated with osteoporosis via hepatokines. However, not all hepatokines involved in bone resorption and osteoporosis. In the present study, we found that the level of serum LECT2 were higher in OP patients than control subjects. These data suggest that serum LECT2 level was closely related to OP, which was consistent with previously reported results of other diseases including obesity, fatty liver, diabetes [15], insulin resistance [29], atherosclerotic [30], and osteoarthritis [31]. We also found that the levels of serum LECT2 were significant negatively associated with lumbar and femoralneck BMD. Our clinical results suggest that LECT2 participates in bone resorption and osteoporosis development, although the direct effect of LECT2 on osteoclast function is not clear.

TC, creatinine, and other metabolic parameters can reflect the physiological state of human liver and kidney. Okumura et al. [15] demonstrated that LECT2 levels correlated with total cholesterol in obesity patients positively. Similarly, in this study we found that LECT2 levels were significant positively associated with TC. Bo et al. [32] reported that higher serum TC levels were associated with greater risk of osteoporosis. Higher lipid levels were associated with higher oxidative stress levels, which could inhibit osteoblast differentiation [33]. Additionally, we found that LECT2 levels were significant negatively associated with creatinine, and lower serum creatinine levels were detected in OP subjects. Similarly, Cui et al. [34] demonstrated that serum creatinine levels were decreased in OP subjects than normal. Huh et

al. [35] also found that serum creatinine was positively associated with BMD in 8648 participants with normal kidney function, and subjects with low serum creatinine got a higher risk for low BMD. The possible explanation was serum creatinine served as a marker of muscle mass, and low skeletal muscle mass was associated with deterioration of BMD. The cause of relationship between LECT2 and creatinine needs to be further explored.

To our knowledge, few specialized clinical markers are available to measure the severity of osteoporosis. The ROC curve analysis showed that serum LECT2 level could be a potential biomarker for male OP patients. The optimal cutoff value for serum LECT2 level for the OP diagnosis was 16.44 ng/mL, suggesting that serum LECT2 levels have clinical significance. Because of the significantly negative correlation between serum levels of LECT2 and BMD in our study, serum LECT2 concentration could be a potential clinical biomarker for the severity of osteoporosis.

In conclusion, to the best of our knowledge, we are the first to describe an increased serum LECT2 level in OP patients. We also found that LECT2 levels were significantly positively associated with total cholesterol and negatively associated with creatinine. Additionally, we found that serum LECT2 level could be a potential biomarker for OP patients. However, due to the limited patient number of this study, further large-scaled perspective study and the pathological role of LECT2 in the development of OP need to be investigated.

Abbreviations

LECT2: leukocyte cell-derived chemotaxin 2; NAFLD: non-alcoholic fatty liver disease; OP: osteoporosis; ELISA: enzyme linked immunosorbent assay; BMD: bone mineral density; ROC: receiver-operating characteristic; IL-1: interleukin-1; IL-6: interleukin-6; TNF: tumor necrosis factor; M-CSF: macrophage colony-stimulating factor; hs-CRP: high-sensitivity C-reactive protein; PG: plasma glucose; ALT: alanine aminotransferase; AST: aspartate amino transferase; TC: total cholesterol, TG: triglycerides.

Declarations

Ethics approval and consent to participate

Written informed consents were obtained from all participants. The study was approved by the Ethics Committee of the Affiliated Hospital of School of Medicine of Ningbo University (Ethics approval code: KY20200202), all methods were performed in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Wen-Ming He, Jiong Chen, Yan-Qing Xie, and Su-Lin Xu. Laboratory experiment and manuscript was finished by Qiang Wang and Feng Xu. All authors read and approved the final manuscript.

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Figures

Figure 1

Concentration of serum LECT2 in control (n = 44) and osteoporosis patients (n = 52). Serum LECT2 levels were significantly increased in osteoporosis patients than that of controls (29.57 ± 15.88 ng/mL VS 19.82 ± 10.75 ng/mL, $**P < 0.01$).

Figure 2

Correlation between serum levels of LECT2 and lumbar BMD in all subjects ($r = -0.347$, $P = 0.001$).

Figure 3

Correlation between serum levels of LECT2 and femoralneck BMD in all subjects ($r=-0.219$, $P=0.033$).

Figure 4

Correlation between serum levels of LECT2 and total cholesterol in all subjects ($r=0.269$, $P=0.008$).

Figure 5

Correlation between serum levels of LECT2 and creatinine in all subjects ($r = -0.205$, $P = 0.045$).

Figure 6

Receiver-operating characteristic (ROC) curve showed the performance of serum LECT2 level in detecting OP patients. The optimal cutoff point was 16.44 ng/mL. The area under the ROC curve was 0.729, $P < 0.01$. The diagnostic efficiency for OP patients reached the highest value with sensitivity and specificity of 98.1% and 47.7%, respectively.