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Reduced serum corin levels in patients with osteoporosis

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ABSTRACT

Background: Corin is a cardiac protease that activates the natriuretic peptides. Corin is also expressed in chondrocytes and bone marrow-derived mesenchymal stem cells that undergo osteogenic differentiation, suggesting a potential role of corin in bone formation and homeostasis.
Methods: To test if corin levels are altered in patients with bone disease, we used ELISA to measure corin and osteocalcin levels in serum samples from healthy controls (n = 134) and patients with osteopenia (n = 53) and osteoporosis (n = 101).
Results: In patients with osteopenia and osteoporosis, serum corin levels were 510 ± 228 and 478 ± 183 pg/ml, respectively, which were significantly lower than that in healthy controls (682 ± 240 pg/ml) (both p values < 0.001). The reduced serum corin levels were found in both male and female patients. In multiple linear regression analysis, bone mineral density was identified as an independent predictor for serum corin levels. In patients with osteopenia and osteoporosis, but not normal controls, a negative correlation was found between serum corin and osteocalcin levels.
Conclusion: Serum corin levels were reduced in patients with osteoporosis and the reduction was associated with high rates of bone turnover. Low serum corin levels may reflect impaired bone homeostasis in patients with osteoporosis.

1. Introduction

Osteoporosis is a skeletal disease characterized by bone mass loss and microarchitectural deterioration of bone tissues, which increases bone fragility and fracture susceptibility [1–5]. The disease affects millions of men and women, especially among aging populations. In elderly women, osteoporosis-associated fractures are a common cause of morbidity and mortality [6]. The cause of osteoporosis is complex, including genetic, metabolic and environmental factors [6–8]. Fundamentally, the disease reflects impaired bone homeostasis, in which bone resorption exceeds bone formation. Clinically, bone mineral density (BMD) is used to assess osteoporosis and its associated risks [2,4]. The BMD measurement, however, provides limited information about dynamic remodeling processes in bone tissues.

Corin is a trypsin-like serine protease identified in cardiac myocytes [9–12]. The biological function of corin is to activate the natriuretic peptides that are important in regulating salt-water balance and blood pressure [13–15]. Recent studies have shown that corin also acts locally in the pregnant uterus to promote trophoblast invasion and spiral artery remodeling, which are critical for promoting uteroplacental blood flow and preventing pregnancy-induced hypertension [16–18].

Unlike trypsin, corin contains a transmembrane domain, which anchors corin on the cell surface [19–21]. The membrane-bound corin undergoes proteolytic shedding, a process that has been reported in many transmembrane proteins [22–24]. To date, soluble corin fragments have been detected in the conditioned medium from cultured cells and human blood samples [23,25–29]. In patients with heart disease, plasma corin levels were found markedly reduced, suggesting that corin defects may contribute to cardiovascular disease [25,30–32].

In addition to the heart, corin is expressed in other tissues including the kidney, skin and bone [12,28,33–35]. By *in situ* hybridization and RT-PCR, corin mRNA was detected in prehypertrophic chondrocytes and perichondrocytes in mouse developing bones and human osteosarcoma-derived cells [12]. More recently, human mesenchymal stem cells (hMSCs) from bone marrow and adipose tissues also were found to express corin mRNAs [36,37]. When the cultured hMSCs underwent osteogenic differentiation, corin mRNA expression was

Abbreviations: BMD, bone mineral density; FN, femoral neck; hMSCs, human mesenchymal stem cells; LS, lumbar spine; TMB, 3,3′,5,5′-tetramethylbenzidine.

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highly up-regulated, suggesting a potential role of corin in regulating mesenchymal stem cell differentiation in chondrogenesis and/or osteogenesis.

If corin plays a role in bone differentiation or remodeling, soluble corin levels in the blood may be used as an indicator for bone homeostasis. To test this hypothesis, we measured serum corin levels in healthy controls and patients with low bone densities. Our data indicate that serum corin levels were reduced significantly in patients with osteopenia and osteoporosis.

2. Materials and methods

2.1. Study population

This study was approved by the ethics committee of the Second Affiliated Hospital of Soochow University, Suzhou, China. All participants gave written informed consent. A total of 154 patients were recruited, including 53 with osteopenia and 101 with osteoporosis, who were treated in the Department of Orthopedics of the hospital. Additional 134 healthy subjects, who underwent routine medical check-ups at the hospital, were recruited as controls. None of the participants were alcohol addicts, on steroids or had histories of heart failure and tumors. The baseline characteristics of the patients and healthy controls are shown in Table 1.

2.2. Clinical assessments

Data collected from control individuals and patients include age, height, body weight and medication history. Body mass index (BMI) was calculated. BMD was determined for the lumbar spine (LS) and the femoral neck (FN) using dual-energy X-ray absorptiometry (DXA, GE Healthcare) [2]. The coefficients of variation (CVs; precision) for LS and FN BMD were 0.7% and 1.2%, respectively. Results were expressed as T-scores [standard deviation (SD) from peak adult BMD]. According to the World Health Organization (WHO) criteria, osteoporosis was considered when LS and/or FN T-scores were ≤ -2.5 SD of the mean value for young adults, and osteopenia was considered when the T scores were ≤ -1.0 SD of the mean value for young adults [38]. Most patients with osteoporosis were treated with vitamin D, calcium, bisphosphonates and anabolic drugs according to the clinical management guidelines.

2.3. Measurement of serum corin

Venous blood samples from fasting participants were collected into tubes without anticoagulants. Serum samples were obtained by centrifugation at 3000 *g* for 15 min, stored at −80 °C and used within one year. Samples from patients and healthy controls were stored for similar lengths of time. An ELISA kit (R&D Systems) was used to measure corin levels in serum [26,39]. In brief, microtiter plates were coated with an anti-corin antibody. Serum samples or recombinant human corin standards were added and incubated at room temperature for 2 h on a rotator (Eppendorf ThermoMixer). After washing with phosphate-buffered saline, a biotin-labeled anti-corin antibody was added and incubated at room temperature for 2 h. After washing, horseradish peroxidase-conjugated streptavidin was added and incubated at room temperature for 20 min. After washing, a horseradish peroxidase substrate (3,3′,5,5′-tetramethylbenzidine, TMB) solution was added and incubated in dark for 30 min. The reaction was stopped by adding H_2SO_4 (2N) and the optical density (OD) in wells was recorded by a spectrometer at the wavelength of 450 nm.

Table 1
Characteristics of healthy controls and patients with osteopenia or osteoporosis.

Characteristics	Control	Osteopenia	Osteoporosis
	(n = 134)	(n = 53)	(n = 101)
Age, mean (SD), (y)	60.3 (11.1)	68.1 (9.6)**	68.1 (16.4)**
Body mass index (BMI, kg/m^2)	24.5 (2.1)	24.6 (1.5)	24.2 (1.6)
Sex, n (%)			
Male	42 (31.3)	1 (1.9)	20 (19.8)
Female	92 (68.7)	52 (98.1)	81 (80.2)
Medical history, n (%)			
Hypertension	0	19 (35.8)	64 (63.4)
Diabetes	0	10 (18.9)	30 (29.7)
Heart disease	0	0	5 (5.0)
Kidney disease	0	0	1 (1.0)
Others	0	6 (11.3)	17 (16.8)

** $p < 0.01$ *vs.* control.

2.4. Measurement of serum osteocalcin

Serum osteocalcin levels were measured using an ELISA kit (Xitang Bio-Tech), according to the manufacturer's instructions. In brief, osteocalcin standards or serum samples were added to pre-coated microtiter plate and incubated for 1 h at room temperature. After washing, a biotin-conjugated anti-osteocalcin antibody was added and incubated at room temperature for 1 h. After washing, horseradish peroxidase-conjugated streptavidin was added and incubated at room temperature for 20 min. After washing, a TMB solution was added and incubated in dark for 15 min. The reaction was stopped by adding H_2SO_4 (2N) and the OD value was read at the wavelength of 450 nm by a plate-reader. Osteocalcin concentrations in samples were calculated based on the osteocalcin standard curve.

2.5. Statistical analysis

The analysis was done using the MedCalc software and the Statistical Analysis Software (SAS Institute). All data were presented as mean ± SD. Differences between groups were analyzed by Student's *t*-test or ANOVA if groups were >2. Multiple linear-regression analysis was performed to identify independent predictors for serum corin levels in patients. Variables in the analysis included sex, age, weight, BMI, hypertension, diabetes and T-score. The correlation between serum corin and osteocalcin levels was analyzed using Pearson's correlation coefficient test. All probabilities were 2-tailed and p values < 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Serum corin levels in patients with osteopenia or osteoporosis

We measured serum corin by ELISA. Serum corin levels in patients with osteopenia or osteoporosis were 510 ± 228 and 478 ±183 pg/ml, respectively, which were significantly lower than that in healthy controls (682 ± 240 pg/ml) (both p values < 0.001) (Fig. 1). The difference between the two patient groups was not statistically significant (Fig. 1).

Previously, plasma corin levels were found higher in males than females [26]. In this study, similar gender differences in serum corin levels were found in healthy controls and patients with osteopenia or osteoporosis. The level in male controls was significantly higher than that of the female controls (894 ± 257 *vs.* 585 ± 154 pg/ml, $p < 0.001$) (Fig. 2A). In male patients, the corin level (643 ± 197 pg/ml) also was significantly higher than that in female patients (465 ± 189 pg/ml, $p < 0.001$) (Fig. 2B).

We divided study subjects into male and female groups and analyzed the data based on the patients' disease stages. In female controls (n = 92) and patients with osteopenia (n = 52) or osteoporosis (n = 81), serum corin levels were 585 ± 154, 507 ± 228 and 438 ± 155 pg/ml, respectively. The differences between the controls and patients with osteopenia ($p < 0.05$) or osteoporosis ($p < 0.001$) were significant (Fig. 2C). The difference between the female patients with osteopenia or osteoporosis also was significant ($p < 0.05$) (Fig. 2C). In the male group, which had only 1 patient with osteopenia, comparison was made between controls (n = 42) and patients with osteoporosis (n = 20). Serum corin levels were significantly lower in the patients