Association of Bone-Related Biomarkers with Femoral Neck Bone Strength

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Research Article

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Abstract

Background: Femoral neck fractures are the worst consequence of osteoporosis (OP), and its early prevention and treatment have become a public health problem.This study aims to investigate the relationship of bone-related biomarkers,femoral neck bone mineral density (BMD) and maximum load (L_{max}),selecting the indicator which can reflect femoral neck bone loss and reduced bone strength.

Methods: A total of 108 patients were recruited from January 2017 to December 2019. Femoral neck BMD, femoral neck $L_{max}$and bone-related biomarkerswere all measured and analyzed.

Results: The expression of femoral neck type I collagen (COL-I)were significantly decreased, whereas other markers were all significantly increased with the decreasing of femoral neck BMD and L_{max}(P<0.05). Among them, serum C-terminal telopeptide of type I collagen (CTX)levels and femoral neck osteopontin (OPN) expression were increased in osteopenia. In multiple linear regression analysis,CTX and OPN were *independent* factorsof femoral neck BMD and L_{max}, whereasCOL-I was *independent* factor affectingL_{max}(P< 0.05). Moreover, CTX was the independent risk factor of OPN and COL-I(P<0.05).

Conclusions: Bone-related biomarkersdo reflect decreased femoral neck bone mass and bone strength. Of them, the CTX increases most significantly in the process of bone loss, and can be used as a surrogate markerof OPN and COL-I for bone loss and reduced bone strength.Early measurement of CTXcouldfacilitate the diagnosis of osteopenia and provide a theoretical basis for delaying the occurrence of femoral neck OP and fragility fractures.

Introduction

Osteoporosis (OP) is a common systemic bone metabolic disease characterized by the reduction of bone mass and microarchitectural deterioration of bone tissue, which ultimately leads to increased bone fragility and fracture risk [1]. In recent years, with the increasing incidence of OP in the world, OP has become an important issue affecting human health [2]. Femoral neck osteoporotic fracture is a serious complication of OP, with the increasing of the aging population, the incidence of femoral neck osteoporotic fractures increases rapidly. Due to the long course, severe symptoms and poor prognosis, femoral neck osteoporotic fractures have attracted increasing attention [3]. Previous studies have shown that femoral neck fractures were in high risk of developing severe complications (e.g., deep vein thrombosis and bed sores) during bed rest, which not only deeply influence the life quality but also cause heavy economic burden on patients and society [4].

Measurement of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) is currently the golden standard for the diagnosis of OP. DXA has many advantages such as rapid detection, wide detection range, and non-invasiveness, it has been widely used in clinical practice [5]. However, DXA is susceptible to bone hyperplasia, fractures and extraosseous calcification at the measurement site. Furthermore, DXA cannot effectively distinguish between trabecular and cortical bone, the different

metabolic rate between trabecular and cortical bone may affect BMD testing results [6]. Several studies have shown that many patients with fragility fractures have normal or slight low bone mass, and thus the diagnosis of OP and the evaluation of fragility fractures through testing BMD alone are not comprehensive [7]. Previous studies have founded that bone-related biomarkers can well reflect bone metabolic activities and the risk of OP [8].

Osteopenia is a critical transition stage from normal bone mass to osteoporosis, and thus the early detection of osteopenia patients could be of great importance for the timely prevention and treatment of osteoporosis [9]. However, many studies have only focused on the detection of osteoporosis, but few on the osteopenia. It is also unclear which indicator can better reflect the early bone loss and change of bone strength. Therefore, the purpose of this study was to find indicators, which can sensitively reflect the early change of bone mass and strength of femoral neck, through investigating the association of femoral neck BMD, femoral neck bone strength and bone-related biomarkers, further providing a theoretical basis for timely discovering the patients with femoral neck osteopenia and preventing the occurrence of femoral neck OP and femoral neck fragility fractures finally.

Materials And Methods

Patients

Patients who underwent total hip replacement at the General Hospital of Western Theater Command were recruited from January 2017 to December 2019. Age, height and body mass index (BMI) were all recorded. The inclusion criteria were as follows: a) total hip replacement surgery was performed; b) there was no obvious contraindications to surgery and no bone metabolic diseases (except OP); c) all patients had femoral neck BMD measured at the ipsilesional side; d) there was no history of mental illness; e) patients or their families signed an informed consent. Exclusion criteria were as follows: a) patients suffered from diseases affecting bone metabolism (e.g., diabetes, thyroid diseases, hyperparathyroidism and rheumatoid arthritis); b) patients received drugs that may affect bone metabolism within 3 months (e.g., glucocorticoids, thyroid drugs, vitamin D supplements and calcium supplements); c) patients who received previous antiosteoporotic treatment; d) severe organ dysfunction; e) severe deformity at the measurement site.

Measurement of BMD

BMD (g/cm^2) of the femoral neck were measured by DXA (Lunar prodigy, GE Medical Systems, Madison, WI, USA). Based on the results of femoral neck BMD, all patients were divided into normal group (T-score $\geq$ -1.0), osteopenia group (T-score < -1.0 and > -2.5), OP group (at least one of the following criteria fulfilled: (a) T-score $\leq$ -2.5; (b) femoral neck fragile fracture) and severe OP group (fragility fracture with a T-score $\leq$ -2.5) according to the WHO diagnostic criteria and the Guidelines for the diagnosis and management of primary osteoporosis (2017).

Analysis of Bone-related biomarkers

Bone turnover markers (BTMs)

Blood samples were collected under fasting condition in the early morning. All samples were immediately centrifuged, and serum was then separated and stored at -80°C until analysis. Serum levels of osteocalcin (OC), type I procollagen N-terminal propeptide (PINP) and C-terminal telopeptide of type I collagen (CTX) were all measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). The detection ranges of serum OC, PINP, and CTX were 2.00–64.00 ng/ml, 2.50–80.00 ng/ml, and 93.75–3000.00 pg/ml, respectively. All procedures were carried out strictly in accordance with the kit instructions.

Biochemical markers

Protein expression of osteopontin (OPN) and type I collagen (COL-I) in the femoral neck was analyzed by Western blot. During the total hip replacement, osteotomy was initially performed 0.5-1.0 cm above the minor trochanter, and then performed under the femoral head to obtain the femoral neck samples. After removing the surrounding soft tissues, the femoral neck was washed with phosphate-buffered saline (PBS) to remove blood and residues. The bone tissue of femoral neck was frozen in liquid nitrogen and ground into a powder for total protein extraction using the bone tissue protein extraction kit (Biomart, Beijing, China). The protein concentration was determined using the BCA protein assay kit (Beyotime, Shanghai, China). 30μg of soluble protein were separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore Corp, Billerica, MA, USA). Then the PVDF membranes were blocked in 5% skim milk for 1 hour at room temperature. Subsequently, the membranes were incubated with the following primary antibodies: anti-β-actin (1:10000, Immunoway, Plano, TX, USA), anti-Osteopontin (1:1000, Millipore Corp, Billerica, MA, USA), anti-Type I Collagen (1:500, Millipore Corp, Billerica, MA, USA) at 4°C overnight. After washing with TBST, all membranes were incubated with secondary antibody (1:10000, Origene, Rockville, MD, USA) for 1 hour at room temperature. After washing with TBST three times, PVDF membranes were detected by UVP Biochemi EC3 Imaging System (UVP, Upland, CA, USA) using chemiluminescence HRP substrate (Millipore Corp, Billerica, MA, USA). The ratio of the optical density of the target protein band to the reference protein band was calculated for statistical analysis.

Compression tests

Tensile experiments were conducted by using MTS model 809 axial/torsional testing system (MTS Systems Corp., USA). Before testing, the superior and inferior planes of the cortical bone samples from the femoral neck were sanded to be parallel to each other. Subsequently, the compression test was performed with a 0.02 mm/s speed until the appearance of obvious peak, and then the maximum load (maximum load, L_{max}) was calculated by the load-displacement curve.

Statistical Analysis

Data with normal distribution were expressed as mean ± standard deviation (SD), and those with non-normal distribution as median and interquartile range. For quantitative data with a normal distribution

and homogeneity of variance, one-way analysis of variance (ANOVA) was performed, with differences among each group assessed using a Bonferroni post hoc test. Data without normally distribution or homogeneity of variance was analysed by the non-parametric Kruskal-Wallis test for statistically significant differences. A chi-squared test was used for categorical variables. Pearson correlation coefficients was used to analyze the relationship of the femoral neck BMD, femoral neck L_{max} and bone-related biomarkers. Multivariate linear regression analyses were performed to identify significant factors that affected the femoral neck BMD and L_{max}. The receiver operating characteristic curve (ROC) analyse was used to evaluate the diagnostic value of indexes for femoral neck osteopenia and OP. $P < 0.05$ indicated that the difference was statistically significant. The statistical analysis was performed using IBM SPSS Statistics 25.0.

Results

General information of the patients

A total of 108 patients were selected in this study with an average age of 61.02 ± 10.84 years and an average BMI of 24.55 ± 3.41 kg/cm^2. Subjects with severe osteoporosis were significantly older than those with either normal BMD or osteopenia ($P = 0.000$). The average age of the subjects with osteopenia and osteoporosis were significantly higher compared with the normal BMD ($P = 0.000$). There were no significant differences in age among other groups ($P > 0.05$). The femoral neck T-score, BMD and L_{max} in the severe OP group were all significantly lower than those in the normal, osteopenia and OP groups ($P = 0.000$). The femoral neck T-score, BMD and L_{max} in the OP and osteopenia groups were significantly lower than those in the normal group ($P < 0.05$). The differences among the other groups were not statistically significant ($P > 0.05$). There were no significant differences in the ratio of men to women and BMI among the groups ($P > 0.05$) (Table 1).

Table 1
Comparison of general clinical information of the patients in each group

Parameters	Normal (n = 27)	Osteopenia (n = 27)	OP (n = 27)	Severe OP (n = 27)	*P*-value
Gender (male/female)	6/21	7/20	6/21	5/22	0.934
Age (years)	51.48 ± 10.71	60.30 ± 6.03*	63.00 ± 9.05*	69.30 ± 8.97*#	0.000
BMI (kg/cm^2)	24.85 ± 3.57	24.76 ± 3.89	24.88 ± 2.78	23.72 ± 3.37	0.545
Femoral neck BMD (g/cm^2)	0.94 ± 0.12	0.80 ± 0.10*	0.77 ± 0.09*	0.61 ± 0.10*#▲	0.000
Femoral neck T-score	0.00 ± 0.90	-1.50 ± 0.48*	-1.75 ± 0.75*	-2.91 ± 0.40*#▲	0.000
Femoral neck L_{max} (KN)	3.00 ± 0.52	1.67 ± 0.46*	1.12 ± 0.26*	0.59 ± 0.19*#▲	0.000
Data are presented as the mean ± SD for age, BMI, femoral neck BMD, femoral neck T-score and femoral neck L_{max}					
* P < 0.05, compared with normal group; # P < 0.05, compared with osteopenia group; ▲ P < 0.05, compared with osteoporosis group.					
OP osteoporosis, BMI body mass index, BMD bone mineral density					

Comparison of serum BTMs

Serum CTX and OC levels in the severe OP group were significantly higher than that in the normal and osteopenia groups, the levels of serum PINP were significantly increased compared with the normal group (P = 0.000). Serum OC levels in the OP group were significantly increased than those in the normal and osteopenia groups, the levels of serum CTX and PINP were significantly increased compared with the normal group (P = 0.000). Serum CTX levels in the osteopenia group were significantly higher than that in the normal group (P = 0.000). The differences of the other groups were not statistically significant (P > 0.05) (Table 2).

Table 2
Comparison of serum BTMs levels in each group

Parameters	Normal (n = 27)	Osteopenia (n = 27)	OP (n = 27)	Severe OP (n = 27)	*P*-value
CTX (ng/ml)	0.29 ± 0.14	0.52 ± 0.16*	0.65 ± 0.20*	0.78 ± 0.23*#	0.000
PINP (ng/ml)	44.00 ± 12.55	48.30 ± 15.95	62.11 ± 21.04*	65.04 ± 23.73*	0.000
OC (ng/ml)	8.33 ± 4.18	10.41 ± 4.67	17.70 ± 8.26*#	18.75 ± 10.45*#	0.000
Data are presented as the mean ± SD for CTX, PINP and OC					
* P < 0.05, compared with normal group; # P < 0.05, compared with osteopenia group; ▲ P < 0.05, compared with OP group					
OP osteoporosis, CTX C-terminal telopeptide of type I collagen, PINP type I procollagen N-terminal propeptide, OC osteocalcin					

Comparison of OPN and COL-I protein expression

Expression of OPN in the severe OP and OP groups was significantly increased compared with the normal and osteopenia groups (P<0.05). Expression of COL-Iin the severe OP and OP groups was significantly lower than that in the normal and osteopenia groups (P<0.05). Expression of OPN in the osteopenia group was significantly increased compared with the normal group (P<0.05). The differences of the other groups were not statistically significant (P > 0.05) (Fig.1.).

Correlation of the femoral neck BMD, femoral neck L_{max} and other parameters

Pearson correlation analysis results showed that femoral neck BMD and femoral neck L_{max} were negatively correlated with serum BTMs and femoral neck OPN (femoral neck BMD: r = -0.531~-0.301, P< 0.01; femoral neck L_{max}: r = -0.644~-0.353, P = 0.000), whereas them were both positively correlated with femoral neck COL-I (femoral neck BMD: r = 0.402, P = 0.000; femoral neck L_{max}: r = 0.527, P = 0.000). Among these markers, serum CTX had the strongest correlation with femoral neck BMD and femoral neck L_{max} (femoral neck BMD: r = -0.531, P = 0.000, femoral neck L_{max}: r = -0.660, P = 0.000) (Table 3).

Table 3 Pearson correlation coefficients of femoral neck BMD, femoral neck L_{max} and other parameters

Parameters		Femoral neck BMD	Femoral neck L_{max}	OC	PINP	CTX	COL-I
Femoral neck L_{max}	*r*	0.655					
	P	0.000					
OC	*r*	-0.352	-0.435				
	P	0.000	0.000				
PINP	*r*	-0.301	-0.353	0.349			
	P	0.002	0.000	0.000			
CTX	*r*	-0.531	-0.660	0.394	0.497		
	P	0.000	0.000	0.000	0.000		
COL-I	*r*	0.402	0.527	-0.246	-0.259	-0.401	
	P	0.000	0.000	0.010	0.007	0.000	
OPN	*r*	-0.481	-0.644	0.419	0.355	0.487	-0.415
	P	0.000	0.000	0.000	0.000	0.000	0.000

CTX C-terminal telopeptide of type I collagen, PINP type I procollagen N-terminal propeptide, OC osteocalcin, OPN osteopontin, COL-Ⅰ type I collagen

Multiple linear regression analysis

The femoral neck BMD and L_{max} were used as dependent variable, respectively, and serum BTMs, femoral neck biochemical markers, age, BMI and gender were all included as independent variables in multivariate linear regression models. Multivariate linear regression analysis revealed that the femoral neck BMD was negatively correlated with femoral neck OPN protein expression (β = -0.220, P = 0.025) and serum CTX levels (β = -0.331, P = 0.001). After adjusting age, gender and BMI, we found that femoral neck BMD was negatively correlated with CTX (β = -0.284, P = 0.004) and OPN (β = -0.203, P = 0.037)(Table 4).

As shown in table 4, femoral neck L_{max} was negatively correlated with OPN (β = -0.355, P = 0.000) and CTX (β = -0.357, P = 0.000), whereas it was positively correlated with COL-I (β = 0.149, P = 0.024) after adjusting age, gender and BMI. Among above indexes, CTX had the strongest correlation with femoral neck BMD and femoral neck L_{max} (P < 0.01) .

Table 4 Multiple linear regression analysis of femoral neck BMD, femoral neck L_{max} and other parameters

Model	Parameters	Femoral neck L_{max}			Femoral neck BMD		
		S.E.	*β*	*P*-value	*S.E.*	*β*	*P*-value
Unadjusted model	OC	0.008	-0.103	0.145	0.002	-0.096	0.289
	PINP	0.004	0.055	0.445	0.001	0.016	0.860
	CTX	0.308	-0.396	0.000	0.059	-0.331	0.001
	OPN	0.235	-0.337	0.000	0.045	-0.220	0.025
	COL-I	0.222	0.217	0.002	0.043	0.158	0.078
Adjusted model	OC	0.008	-0.068	0.301	0.002	-0.036	0.684
	PINP	0.003	0.101	0.138	0.001	0.083	0.361
	CTX	0.283	-0.357	0.000	0.057	-0.284	0.004
	OPN	0.221	-0.355	0.000	0.045	-0.203	0.037
	COL-I	0.208	0.149	0.024	0.042	0.100	0.255
Adjusted for age, BMI and sex							
CTX C-terminal telopeptide of type I collagen, PINP type I procollagen N-terminal propeptide, OC osteocalcin, OPN osteopontin, COL-Ⅰ type I collagen							

The association of BTMs and biochemical markers was further performed (Table 5). After adjusting age, gender and BMI, we found that CTX was negatively correlated with COL-I (β = -0.275, P = 0.012) and positively correlated with OPN (β = 0.295, P = 0.003).

Table 5 Multiple linear regression analysis between serum BTMs and femoral neck biochemical markers

Model	Parameters	OPN			COL-I		
		S.E.	*β*	*P*-value	*S.E.*	*β*	*P*-value
Unadjusted model	OC	0.003	0.251	0.007	0.004	-0.093	0.350
	PINP	0.002	0.100	0.302	0.002	-0.060	0.565
	CTX	0.424	0.338	0.001	0.130	-0.334	0.002
Adjusted model	OC	0.004	0.192	0.039	0.004	-0.041	0.686
	PINP	0.002	0.042	0.664	0.002	0.000	0.998
	CTX	0.123	0.295	0.003	0.131	-0.275	0.012
Adjusted for age, BMI and sex							
CTX C-terminal telopeptide of type I collagen, PINP type I procollagen N-terminal propeptide, OC osteocalcin, OPN osteopontin, COL-Ⅰ type I collagen							

ROC curve analysis

To assess the potential diagnostic value of serum BTMs and femoral neck biochemical markers, the ROC curve analysis was performed. As shown in Table 6 and Fig. 2, the AUC of CTX and OPN were significantly higher than PINP and OC ($P < 0.05$). The differences of other markers were not statistically significant ($P > 0.05$). Further more, ROC analysis of each marker in diagnosing femoral neck osteoporosis was further performed. We found that the AUC of OPN and COL-I were greater than PINP and OC ($P < 0.05$). The differences of other markers were not statistically significant ($P > 0.05$) (Table 7, Fig. 2.).

Table 6
The value of each marker in diagnosing femoral neck osteopenia

Parameters	AUC	*95% CI*	*S.E.*	Cut-off	Sensitivity (%)	Specificity (%)	*P*-value
OC	0.741	0.648 ~ 0.834	0.047	15.74	0.481	0.963	0.000
PINP	0.695	0.595 ~ 0.794	0.051	58.99	0.494	0.926	0.003
CTX	0.914	0.852 ~ 0.976	0.032	0.41	0.864	0.889	0.000
COL-I	0.802	0.701 ~ 0.904	0.052	0.63	0.975	0.963	0.000
OPN	0.908	0.851 ~ 0.965	0.029	0.14	0.901	0.852	0.000

AUC the area under the curve, CTX C-terminal telopeptide of type I collagen, PINP type I procollagen N-terminal propeptide, OC osteocalcin, OPN osteopontin, COL-Ⅰ type I collagen

Table 7
The value of each marker in diagnosing osteoporosis

Parameters	AUC	*95% CI*	*S.E.*	Cut-off	Sensitivity (%)	Specificity (%)	*P*-value
OC	0.767	0.672 ~ 0.862	0.048	16.98	0.611	0.926	0.000
PINP	0.726	0.628 ~ 0.824	0.050	61.26	0.593	0.889	0.000
CTX	0.842	0.769 ~ 0.914	0.037	0.59	0.685	0.852	0.000
COL-I	0.916	0.861 ~ 0.972	0.028	0.46	0.852	0.907	0.000
OPN	0.891	0.819 ~ 0.962	0.036	0.44	0.870	0.907	0.000

AUC the area under the curve, CTX C-terminal telopeptide of type I collagen, PINP type I procollagen N-terminal propeptide, OC osteocalcin, OPN osteopontin, COL-□ type I collagen

Discussion

Along with an increasing aging population, the number of patients with OP and fragility fractures gradually increase. It is estimated that there will be 5.99 million patients with osteoporotic fractures by 2050 in China [10]. Recently, femoral neck fragility fractures have received more and more attention due to the high rate of morbidity and mortality [4].

DXA is currently the golden standard for the diagnosis of OP, but it is not sensitive to monitor early changes of BMD, the change of bone loss can be identified by DXA only when bone loss reaches a certain level [11]. Moreover, OP is characterized by insidious onset and slow progression and is easily to be ignored, several patients already missed the best opportunity for treatment before diagnosed, leading to a poor prognosis.

Femoral neck L_{max} is defined as the maximum force that femoral neck can tolerate before fracture, and it is an important quantity for the mechanical property [12]. In our study, we founded that the femoral neck L_{max} was significantly decreased with the decreasing of femoral neck BMD, indicating that femoral neck bone loss was linked to reduced femoral neck BMD. The reduced femoral neck BMD may be the trigger for the increased femoral neck bone fragility and osteoporotic fractures.

OPN is one of the main non-collagen proteins in bone tissue, it is mainly involved in inhibiting bone mineral deposition and accelerating bone loss [13]. Multiple studies have shown that OPN is significantly increased in patients with OP compared with patients with normal bone mass [14]. COL-I is the major components of organic bone matrix and has an important effect on bone mechanical strength. Haynl et

al. [15] showed that the decline of COL-I protein expression can lead to an increase in the risk of OP. Although several studies have shown that OPN and COL-I both play an important role in the incidence and development of OP [16–18], there are few studies on the changes of OPN and COL-I protein expression in the femoral neck under different femoral neck BMD. The effects of OPN and COL-I on the bone loss and reduced bone strength of femoral neck are still unclear.

In our study, we showed that COL-I protein expression of femoral neck in the severe OP and OP groups was significantly lower than that in both normal group and osteopenia group ($P < 0.05$).

Furthermore, we found that protein expression of COL-I in femoral neck was positively correlated with the femoral neck L_{max} ($\beta = 0.149$, $P = 0.024$), indicating that the reduced bone strength of femoral neck is related to COL-I protein expression, and the decreased expression of COL-I may be the cause of femoral neck OP and femoral neck fragility fractures. At the same time, we found that with the gradual decreases of femoral neck BMD and L_{max}, expression of OPN gradually increased ($P < 0.05$). Furthermore, OPN was negatively correlated with the femoral neck BMD and L_{max} ($P < 0.05$), indicating that the increased OPN might be the independent risk factor for the decline of femoral neck bone mass and bone strength. In addition, we are currently analyzing the interactions among the biochemical markers, spatial structure and biomechanical properties of femoral neck in order to fully elucidate the impact of the biochemical markers on the overall biomechanics of the femoral neck and their relationship with femoral neck fractures.

Serum BTMs can reflect the overall status of bone metabolism and detect the early changes of bone mass, they play an important role in guiding OP clinical diagnosis and treatment [19, 20]. However, there are multiple BTMs and which indicator can better reflect the early reduction of femoral neck bone mass and bone strength is still unknown. The results of our study showed that serum CTX, PINP, and OC levels in the severe OP and OP groups were significantly higher than those in the normal group ($P = 0.000$). These results are consistent with previous studies [21] showing that serum CTX, PINP and OC levels can reflect the changes in bone metabolism and bone mass, facilitating the early diagnosis and treatment of OP. We also found that serum CTX levels has changed significantly at the stage of femoral neck osteopenia, which indicates that serum CTX are more sensitive to the decline of femoral neck BMD, the measurement of serum CTX is conducive to early prevention and treatment of femoral neck OP. There are also some studies showing that with the decreases of BMD, serum OC levels gradually decrease [22]. Julien et al. [23] found that there was no significant difference in serum OC levels between OP patients and non-OP patients ($P > 0.05$). The discrepancy could be considered that all patients in previous studies were male who had relatively high peak bone mass, slow bone loss, and low conversion state of bone metabolism. Thus, the upward trend of serum OC levels in male patients is not significant.

After adjusting age, BMI and gender, multivariate linear regression analysis revealed that serum CTX was negatively correlated with femoral neck BMD and L_{max} ($P < 0.01$), indicating that the increased serum CTX was the independent risk factor for the reduced, early measurement of CTX can better reflect the loss of

femoral neck bone mass and bone strength. More than that, CTX could not only be used to monitor the early reduction of femoral neck bone mass and bone strength, and also be helpful to find patients with femoral neck osteopenia.

This study showed the cut-off value of serum BTMs for femoral neck osteopenia and OP. However, it is difficult to compare these results with previous studies because serum BTMs levels are dependent on the detection methods [24, 25]. Hu et al. [26] used electrochemiluminescence immunoassay to measure serum CTX levels and showed that the cut-off values of serum CTX, PINP, OC levels for diagnosing male OP were 0.38ng/mL, 42.43ng/mL, and 16.57ng/mL, respectively. The cut-off values for diagnosis of female OP were 0.21 ng/mL, 32.90 ng/mL, and 13.90 ng/mL, respectively. Therefore, different detection methods can lead to differences in the level of BTMs and the cut-off value for the diagnosis of osteopenia and OP needs to be further investigated. In addition, we found that the AUC of CTX and OPN for femoral neck osteopenia was significantly higher than that of PINP and OC, the AUC of OPN and COL-I for femoral neck OP was significantly higher than that of PINP and OC ($P < 0.05$), suggesting that CTX and OPN may have higher diagnostic value in femoral neck osteopenia, OPN and COL-I may have higher diagnostic value in femoral neck OP.

The results of the above studies confirmed that CTX, OPN and COL-I all can well reflect the early change of femoral neck bone mass and bone strength, and help to early identify patients at high risk for the femoral neck osteopenia and OP. However, we should extract the femoral neck bone tissue from patients in the clinical work, it would not only not only increase patient suffering but also limit clinical use because the detection process is tedious, subject to error and time-consuming, although OPN and COL-I both can directly reflect reduced femoral neck bone mass and bone strength. In our study, we found that CTX had the strongest relationship with femoral neck BMD and L_{max} compared with OPN and COL-I, suggesting that CTX can better reflect the decline of femoral neck bone mass and bone strength. Furthermore, we found that CTX had significantly correlated with OPN and COL-I, indicating that CTX could be considered a surrogate marker for OPN and COL-I. We can be aware of the early change of femoral neck bone mass and bone strength from patients.

Despite the significance of our findings, our study still has some limitations that are worthy of mention. We plan to enlarge the sample size in the following study and standardize the examination process of each indicator to obtain more reliable achievements and to guide clinical practice more precisely in further.

Conclusions

In summary, compared with other indicators, serum CTX was more sensitive to the early change of bone mass and bone strength of femoral neck, and could be considered as a surrogate marker for OPN and COL-I. Therefore, early measurement of serum CTX not only help to reflect early changes of femoral neck bone mass and bone strength but also facilitate the diagnosis of osteopenia and provide a theoretical basis for delaying the occurrence of femoral neck OP and fragility fractures.

Abbreviations

ANOVA: Analysis of variance; AUC:The area under the curve; BMD:Bone mineral density; BMI:Body mass index; BTMs:Bone turnover markers; COL-I:Type I collagen; CTX:C-terminal telopeptide of type I collagen; DXA:Dual energy X-ray absorptiometry; ELISA:Enzyme-linked immunosorbent assay; OC:Osteocalcin; OP:Osteoporosis; OPN:Osteopontin; PBS:Phosphate-buffered saline; PINP:Type I procollagen N-terminal propeptide; PVDF:Polyvinylidene fluoride; ROC:Receiver operating characteristic curve

Declarations

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Not applicable.

Authors' contributions

XN, CY, and WW designed the study and collected data. LYM, BC, and XQYanalyzed the data. XN, CYwrote the manuscript. LDand XW reviewed and edited the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors have declared that no competing interest exists.

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Availability of data and materials

The data that support the findings of this study are available from theGeneral Hospital of Western Theater Command but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Ethics Committee of heGeneral Hospital of Western Theater Command.

Ethics approval and consent to participate

All participants signed a written informed consent form, and the study protocol was approved by The General Hospital of Western Theater Command. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing financial interests.

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Figures

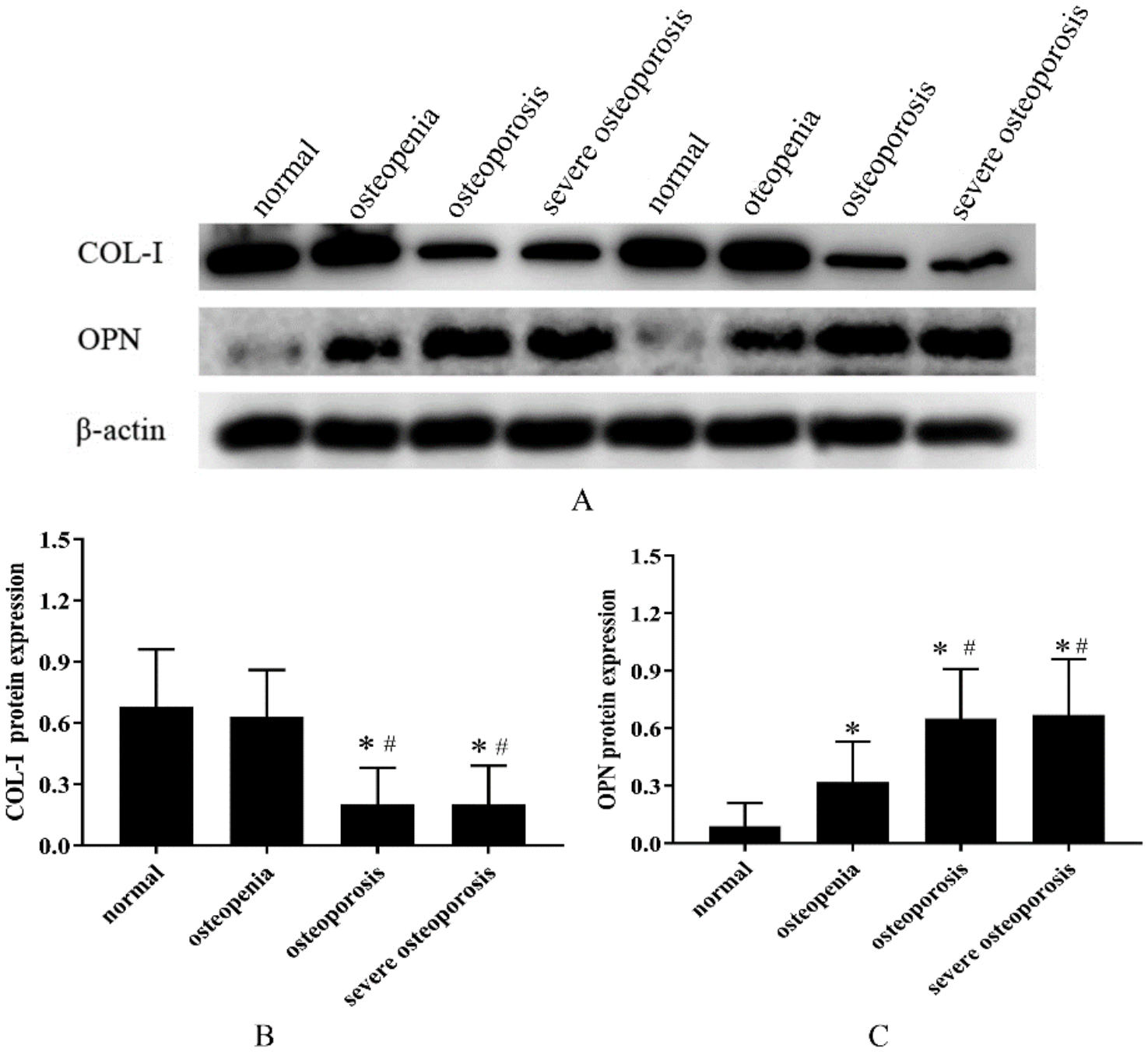

Figure 1

Expression of COL-I and OPN in femoral neckwith different femoral neck BMD A: Western blot analysis of COL-I and OPN expression in different groups; B: Comparison of COL-I expression among different

groups; C: Comparison of OPN expression among different groups *P<0.05, compared with normal group; #P<0.05, compared with osteopenia group COL-I type I collagen, OPN osteopontin

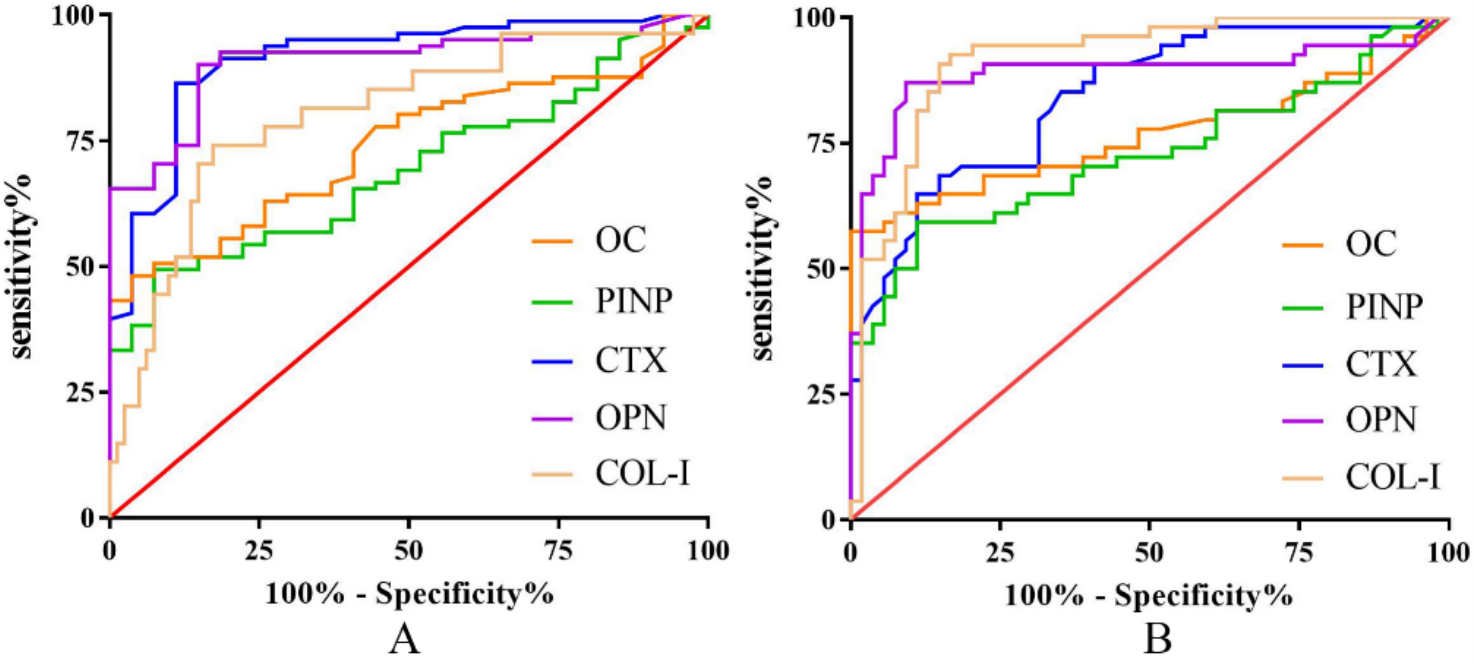

Figure 2

ROCanalysis for each marker A. ROC curve of each marker for diagnosis of femoral neck osteopenia; B. ROC curve of each marker for diagnosis of femoral neck osteoporosis. CTX C-terminal telopeptide of type I collagen,PINP type I procollagen N-terminal propeptide, OC osteocalcin, OPN osteopontin, COL-Ⅰtype Icollagen