

Table		
Demographic and clinical characteristic of post-menopausal women		
Variable	Vitamin D and calcium (n=175)	Diet rich in vitamin D and calcium (n=175)
Age, year, mean \pm SD ^a	47.2 \pm 2.4	46.1 \pm 1.9
Post-menopausal women with history of fracture, n (%) ^b	72 (41%)	69 (39%)
Weight (in kg), mean \pm SD ^a	68.3 \pm 4.4	67.1 \pm 3.2
Height, cm, mean \pm SD ^a	186 \pm 4.3	184 \pm 3.9
Body mass index (kg/m ²) ^a	24.4 \pm 1.7	23.8 \pm 1.3
Use of steroid medication, n ^b	62	67
Bone mineral density, mean \pm SD ^a		
Spine	0.8 \pm 0.2	0.7 \pm 0.2
Neck	0.6 \pm 0.1	0.6 \pm 0.1
Hip	0.5 \pm 0.2	0.4 \pm 0.9
Incidences of bone trauma, n ^b		
Yes	90	86
Women taking osteoporosis medication, n ^b	43	45
Values are presented as mean (SD) or as absolute number (%). N = Total number of subjects, n = number of subjects in each category. ^a p>0.05 indicate no statistical significant difference (calculated by Un-paired test); ^b p>0.05 indicate no statistical significant difference (calculated by Chi-square test).		

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Serum osteocalcin and urinary free deoxypyridinoline as potential risk factors in predicting the prevalence of bone trauma among the post-menopausal Chinese women

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Anqing Jiang, Yijie Liu, Xuefeng Li, Jie Chen, Heng Wang, Huilin Yang and Weimin Jiang

Department of Orthopedics, The First Affiliated Hospital of Soochow University, Suzhou 215006, China.

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Abstract

This study was designed to understand whether the post-menopausal Chinese women (n=175) receiving tablet containing vitamin D (500 IU) and calcium (500 mg) had lower incidence of bone fracture compared to the post-menopausal Chinese women (n=175) receiving a diet rich in calcium, vitamin D, and protein (milk, cheese, and yogurt, soybeans, spinach, fish including fatty fish, cheese, egg). This study assessed whether the levels of serum osteocalcin and urinary free deoxypyridinoline could be used as predictors of early bone trauma during post-menopausal period. After randomization, subjects were followed-up for up to 3 years to capture required data. The results suggested that therapeutic intervention (vitamin D and calcium) does not predict bone fracture among the post-menopausal Chinese women. However, correlation analysis revealed that the decreased level of serum osteocalcin and urinary free deoxypyridinoline were associated with higher incidence of fracture. The results suggest that the low level of serum osteocalcin and urinary free deoxypyridinoline cause increase susceptibility of fracture among the post-menopausal Chinese women.

Introduction

Osteoporosis is one of the leading causes of morbidity and becoming a major public health problem worldwide and its prevalence is increasing. The prevalence of osteoporosis in China remains low compared to that in the White population (Wang, 2009; Zhang, 2014; Tsai, 1996).

It has been reported that the higher levels of bone formation and resorption markers are significantly associated with higher bone mineral density loss. In clinical studies, it appears that markers of bone resorption may be useful predictors of fracture risk and bone loss. The association of markers of bone resorption with hip fracture risk in adults is independent of bone mineral density, but a low bone mineral density combined with high bone resorption biomarker may

increases the risk of bone fracture/trauma. However, the predictive value of biomarkers in assessing bone trauma in post-menopausal Chinese women has not yet been confirmed. Bone turnover markers may have a future role in the clinical management of osteoporosis.

It is not known whether the post-menopausal Chinese women receiving tablet containing vitamin D and calcium had lower incidence of bone fracture compared to the post-menopausal Chinese women receiving a diet rich in calcium, vitamin D, and protein, plays any role in predicting the bone fracture among post-menopausal Chinese women. Also, it is not known whether the levels of serum osteocalcin and urinary free deoxypyridinoline could be used as predictors of early bone fracture in post-menopausal Chinese. Therefore, the present interventional clinical trial was designed to understand whether the post-menopausal Chinese with



osteoporosis receiving tablet containing vitamin D (500 IU) and calcium (500 mg) had lower incidence of bone fracture compared to the children receiving a diet rich in calcium, vitamin D, and protein (milk, cheese, and yogurt, soybeans, spinach, fish including fatty fish, cheese, egg). This study also assessed whether serum osteocalcin and urinary free deoxyypyridinoline could be used as the predictors of early bone fracture in post-menopausal Chinese.

Materials and Methods

Subjects

In this pilot study, post-menopausal Chinese women with osteoporosis or history of bone fractures were enrolled. A total of 389 women were entered into the screening phase. Of these, a total of 350 post-menopausal Chinese women during January 2012 to December 2016 who were visited in the hospital at the time of their consultation were enrolled and were follow-up for up to 3 years to capture required data. All participants underwent laboratory tests including bone mineral density (measured using Dual-energy X-ray absorptiometry [DXA, previously DEXA]) or any other investigation as required investigator to confirm their eligibility for this study. Ethics approval was obtained from institutional review board of Soochow University (SCU/IRB/2014/893). All enrolled women were randomized to receive tablet containing vitamin D (500 IU) and calcium (500 mg) or diet rich in calcium, vitamin D, and protein (milk, cheese, and yogurt, soybeans,

spinach, fish including fatty fish, cheese, egg), and undergone study follow-up for up to 3 years in allocation ratio of 1:1. Blood samples (5 mL) and urine sample were obtained from each enrolled subjects for estimation of serum osteocalcin and urinary free deoxyypyridinoline, respectively. Blood was collected into a tube containing potassium ethylenediamine tetraacetic acid (EDTA) and stored at less than -80°C. Venous blood sample of all the recruited subjects was drawn in syringe taking all aseptic precautions. The sample taken was kept in plain vial at room temperature before sending into the laboratory.

Non-fasting samples of urine (10 mL) were collected in morning (before 9 am, first-void urine samples). Borate (1 g/L) was added as preservative to urine samples to prevent bacterial growth, and samples were stored at -20°C until analyzed.

The sample was tested for serum level of serum osteocalcin by ELISA kit and urinary free deoxyypyridinoline by and deoxyypyridinoline (DPD) ELISA Kit, respectively.

Statistical analysis

Normality of data was checked using KM or SW test, and interpretation is solely depends on the results of p value of SW/KM test which confirm the normality of the data. Quantitative variable was presented as Mean (Standard deviation), and analyzed by parametric/non-parametric statistical test based number of comparison group and distribution of data, using 2 sided statistical tests. Categorical variables were presented as absolute

Table I

Demographic and clinical characteristic of post-menopausal women

Variable	Vitamin D and calcium (n=175)	Diet rich in vitamin D and calcium (n=175)
Age, year, mean \pm SD ^a	47.2 \pm 2.4	46.1 \pm 1.9
Post-menopausal women with history of fracture, n (%) ^b	72 (41%)	69 (39%)
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Values are presented as mean (SD) or as absolute number (%). N = Total number of subjects, n = number of subjects in each category. ^ap>0.05 indicate no statistical significant difference (calculated by Un-paired test); ^bp>0.05 indicate no statistical significant difference (calculated by Chi-square test)

Table II			
Percentage change in serum osteocalcin and urinary free deoxyypyridinoline from baseline to endpoint in post-menopausal Chinese women			
Variable	Postmenopausal women with bone trauma/fracture (n=176) (Median)	Postmenopausal women with no bone trauma/fracture (n=174) (Median)	P values (between group comparison)
Serum osteocalcin (ng/mL)			
Baseline (median)	10.3	23.3	p<0.001 ^a
Difference (95% CI)		13.0 (9.8-24.21)	
Endpoint (median)	11.1 ^c	29.1 ^c	p<0.001 ^b
Difference (95% CI)		18.0 (10.3-30.8)	
Proportion of subjects with low levels	93%	10%	
Proportion of subjects with normal levels	7%	90%	
Urinary free deoxyypyridinoline (ng/mL)			
Baseline (median)	3.8	7.0	p<0.001 ^a
Difference (95% CI)		3.2 (2.1-7.4)	
Endpoint (median)	4.1 ^c	9.4 ^c	p<0.005 ^b
Difference (95% CI)		5.3 (4.3-10.2)	
Proportion of subjects with low levels	75%	70%	
Proportion of subjects with normal levels	25%	30%	

^ap value calculated using unpaired t-test; ^bp value calculated using chi-square test; ^cp<0.05 for within group comparison; Values are presented as Median

number and/or percentage of subjects in each category, and analyzed by Chi-square or fisher exact test based on size of data, using 2 sided statistical tests. Correlations of between the levels of serum osteocalcin and urinary free deoxyypyridinoline with the incidences of bone trauma/fracture were analyzed using Pearson's correlation/regression models. Data from each patient was coded and analyzed using GraphPad Prism statistical analysis software (version 6.0).

Results

Of enrolled women, a total of 176 women had at least 1 bone fracture/trauma during 3 year study follow-up period, and remaining 174 women did not experienced any fracture/trauma (Table I).

This study results showed that there was no statistically significant difference in bone fracture/trauma incidence rate between the post-menopausal Chinese women who received tablet containing vitamin D and calcium, and the post-menopausal Chinese receiving a diet rich in calcium, and vitamin D (Table I). It was noted that the levels of serum osteocalcin and urinary free deoxyypyridinoline were significantly lesser in sub-group who had higher incidence of bone trauma/fracture, irrespective of therapeutic intervention (calcium and vitamin D).

Table III		
Association between serum osteocalcin, urinary free deoxyypyridinoline with the incidences of bone trauma in post-menopausal Chinese women		
Key biomarkers	Incidences of bone trauma (n= 176)	
	Beta-coefficients	p value
Serum osteocalcin	-0.7	<0.005
Urinary free deoxyypyridinoline	-0.6	<0.005

Analysis was performed using Pearson's correlation/regression models

In this study, it has been observed that the levels of serum osteocalcin (11.1 vs. 29.1 ng/mL) and urinary free deoxyypyridinoline (4.1 vs. 9.4 ng/mL) were significantly lesser in subject who experienced bone trauma/fracture as compared to subject with no bone trauma/fracture during study period (Table II). Correlations analysis revealed that the decreased level of serum osteocalcin (11.1 ng/mL) and urinary free deoxyypyridinoline (4.1 ng/mL) were associated with higher incidence of bone trauma/fracture (Table III). This indicates that the low levels of serum osteocalcin and urinary free deoxyypyridinoline causes increase susceptibility of bone trauma/fracture among post-

menopausal Chinese women. In majority of patients who had no bone trauma/fracture, the levels of serum osteocalcin and urinary free deoxypyridinoline were in normal range (Table II).

Discussion

To the best of knowledge, this was the first interventional clinical trial to understand whether the postmenopausal Chinese women with osteoporosis receiving tablet containing vitamin D and calcium had lower incidence of bone trauma compared to the postmenopausal Chinese women receiving a diet rich in calcium and vitamin D. This study results showed that there was no statistical significant difference in incidence of bone trauma between postmenopausal Chinese of both the groups (tablet containing vitamin D (51%) and calcium or diet rich in calcium, and vitamin D (49%). This study results were in consistent with the previous reports which showed failure in demonstrating a statistical significant benefit after calcium and vitamin D supplementation on fracture risk (Jackson et al., 2006; Grant et al., 2005). A Women's Health Initiative trial (in UK) enrolled postmenopausal European women with or without osteoporosis, and were administered calcium plus vitamin D supplementation, calcium plus vitamin D supplementation increased hip bone mineral density (Jackson et al., 2006). Another randomized study in UK was conducted in postmenopausal European women (Randomized Evaluation of Calcium or vitamin D [RECORD] trial), the study results showed that the calcium and/or vitamin D supplementation did not significantly reduce the incidence of low-energy fracture (Grant et al., 2005). Also, this is the first clinical study to assess whether plasma levels of serum osteocalcin and urinary free deoxypyridinoline could be used as predictors of early bone trauma/fracture risk in postmenopausal Chinese women. Due to high intra-individual and interindividual variability and large discrepancy in normal values of biochemical markers, it is difficult to recognize the individuals who may be at risk of developing bone trauma/fracture risk (Jensen et al., 1997a; Jensen et al., 1997b). In addition to this, there is a large variability between analytical methods and standard from laboratory to laboratory (Jensen et al., 1997a; Jensen et al., 1997b). Authors have concluded that large biological variability in the biochemical markers of bone turnover make them unsuitable for diagnosis for prediction of future bone loss in individual patients (Jensen et al., 1997a; Jensen et al., 1997b). Therefore, standardized method was used and all the assessment was performed at single laboratory in order to avoid any confounding variables. Correlations analysis revealed that the decreased level of serum osteocalcin and urinary free deoxypyridinoline were associated with higher incidence of bone trauma/

fracture in this study. The results of this study is in consistent with the previous reports by Uebelhart et al who suggested that the combination of a single measurement of serum osteocalcin, urinary hydroxyproline and urinary deoxypyridinoline performed in early postmenopausal women was correlated to the rate of bone loss (Uebelhart et al., 1991). In contrast to this, another study showed that biochemical bone markers are not useful in diagnosing postmenopausal osteoporosis (Nawawi et al., 2001); this may be due to high variability in response of enrolled patients.

Moreover, this study results suggested that the low levels of serum osteocalcin and urinary free deoxypyridinoline causes increase susceptibility of bone trauma/fracture among postmenopausal Chinese women. In majority of patients who had no bone trauma/fracture, the levels of serum osteocalcin and urinary free deoxypyridinoline were in normal range. The results of this study suggested that the levels of serum osteocalcin and urinary free deoxypyridinoline could predict the prevalence of bone trauma/fracture in postmenopausal Chinese women.

Since the study was designed as pilot study and conducted at single study center in China (limitation of study). Therefore, the present findings cannot be generalized to the overall Chinese population. Based on the study results, study results encourage for conducting large multi-centric randomized clinical study in future to generalize the findings of this study. The hypothesis of this study for relationship between the levels of serum osteocalcin and urinary free deoxypyridinoline with the incidences of bone trauma/fracture was met. Overall, results showed the low levels of serum osteocalcin and urinary free deoxypyridinoline causes increase susceptibility of fracture among postmenopausal Chinese women. Thus, this study results showed that the levels of serum osteocalcin and urinary free deoxypyridinoline are one of the key predictors of bone trauma/fracture in postmenopausal Chinese women with osteoporosis.

Conclusion

Therapeutic intervention does not predict bone trauma/fracture risk in postmenopausal Chinese women with osteoporosis. However, the lower levels of serum osteocalcin and urinary free deoxypyridinoline can be considered as predictors of fracture risk in postmenopausal Chinese women with osteoporosis.

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Ethical Issue

The study protocol was approved by the ethics committee of Soochow University (SCU/IRB/2014/893), China, and written informed consent was taken from each study subjects. All subjects were educated about the study protocol and the likely benefits to the society.

Conflict of Interest

Authors declare no conflict of interest

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Author Info

Weimin Jiang (Principal contact)
e-mail: joniehiselaklis@yahoo.com

First two authors contributed equally