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Original Article

Correlation analysis for follicle-stimulating hormone and C-terminal cross-linked telopetides of type i collagen in menopausal transition women with osteoporosis

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Abstract: The sudden drop in estrogen in post-menopausal women can lead to osteoporosis. About one in three women aged more then 50 years experienced an osteoporotic fracture. The aim of this study is to explore function of follicle-stimulating hormone (FSH) in bone metabolism and osteoporosis in menopausal transition women. 164 cases of women in menopausal transition were included, and which were divided into three groups, including < 40 years group, 40-50 years group and > 50 years group. All of patients must with empty stomach, and 5 ml blood was collected from median cubital vein between second day to ninth day in menstruation period. Examination of FSH and C-terminal cross-linked telopetides of type I collagen (CTx) was performed by using ECLIA assay. The results indicated that CTx levels were distinguished among the different aged group, with the increasing level following with the increased age. FSH level in both of < 40 years and 40-50 years old women were positively correlated with CTx level ($P < 0.05$). The spearman rank correlation analysis results also showed that there were no significant correlation between CTx level and FSH level in > 50 years old women. There were significant differences for the CTx level between 0-40 mIU/ml group and > 40 40 mIU/ml group in all of the three ages group ($P < 0.05$). In conclusion, it's clinically significant for the combining examination of FSH and CTx in menopausal transition women, which could observe the bone metabolize changes quickly and sensitively, and prevent or therapy the osteoporosis in a further step.

Keywords: Follicle-stimulating hormone, C-terminal cross-linked telopetides of type I collagen, menopausal transition, osteoporosis

Introduction

Osteoporosis has become a kind of common disease and frequently-occurring disease for the elders [1]. Osteoporosis is a disease characterized by a reduction in bone mass associated with enhanced bone fragility with a consequent increase in fracture risk, with fractures occurring from minimal or no trauma [2]. According to the World Health Organization (WHO), osteoporosis occurs when bone mineral density falls to more than 2.5 standard deviations (SD) below the standard reference for maximum bone mineral density of the young adult female [3]. The morbidity of osteoporosis in women is 3 to 8 times higher, and with the earlier incidence compared to in men. The sudden drop in estrogen in post-menopausal women can lead to osteoporosis [4]. About one in three women aged more then 50 years (mainly post-menopausal women) experienced an osteoporotic fracture in their lifetime [5, 6]. All of the above data show that the menopausal transition women are the high risk group for the osteoporosis.

In this study, we discussed the effects of follicle-stimulating hormone (FSH) and Heterogeneous C terminal peptide of type I collagen (CTx) on the diagnosis of women osteoporosis in menopausal transition.

Materials and methods

Subjects

One hundred and sixty-four women in menopausal transition were recruited from August 2011 to November 2012 at the department of

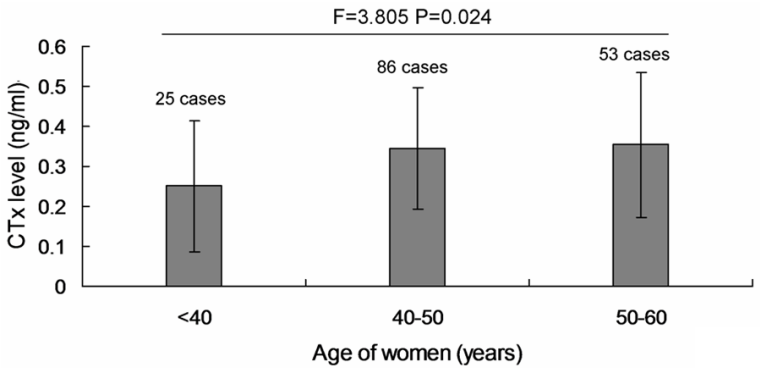

Figure 1. CTx levels in < 40 years old, 40-50 years old and > 50 years old women. The different values among groups were indicated upper of the Figure.

Table 1. CTx levels in different FSH level of < 40 years old women

FSH	Cases	Mean	Standard deviation	95% CI		Minimum	Maximum
				Lower	Upper		
0-40	16	0.2106	0.1309	0.1408	0.2804	0.07	0.59
40~	9	0.3236	0.1983	0.1711	0.4760	0.12	0.71
Total	25	0.2513	0.1640	0.1836	0.3190	0.07	0.71

gynaecology and obstetrics. The median age is 47.38 ± 6.03 years (range from 33 to 60 years). The diagnosis of menopausal transition was defined as the period from ovarian function beginning recession to last time of menstruation [7]. The present study was approved by the Ethics Committee of China Medical University. All patients investigated gave their informed consent. All of these subjects were divided into three group with the interval of 10 years, including less then 40 years group (< 40, 25 cases), range from 40 to 50 years group (40~50, 86 cases) and rage from 50 to 60 group (50~60, 53 cases).

Exclusive criteria

Patients were excluded with a series of diseases. (1) Patients with The female hormone dependent tumor and serious Breast diseases; (2) Some disease which may affect Bone turnover biochemical indicator, including hyperthyreosis, hyperparathyroidism, arthritis deformans, Primary myeloma, bone tumor, bone tumor, spinal angioma, chronic diarrhea, and ect; (3) Application of drugs affecting bone metabolism, including vitamin D, estrogen, thyroid hormone, corticosteroid hormone, heparin, immunosuppressant, calcic preparation, antiepileptic drugs, fluoride, diureticum, and ect; (4) Combing with cardiovascular disease, liver disease, kidney disease, Bone and joint disease and serious hematological system disease and mental disease, and ect. (5) Non natural menopause patients (undergo the uterus or bilateral accessory resection operation).

This study was approved by the ethics committee of Zhejiang University. All of the subjects included in this study have been given their consents and approved this study.

Sample collection

For all of the subjects, who must with empty stomach, and 5 ml blood was collected from the median cubital vein between the second day to the ninth day in menstruation period. The collected blood was centrifuged with the speed of 3000 round/min, and the serum was stored at -80°C refrigerator for the latter examination.

Electrochemiluminescence immunoassay assay (ECLIA)

The examination of FSH and CTx was performed by using ECLIA assay. All of the experimental processes were performed according to the instruments of the kits (Roche, Berlin, Germany) and the method described previously [8]. The serum was detected under the full automatic chemiluminescence immunoassay analyzer (Roche Modular E170, Berlin, Germay).

Statistical analysis

Statistical analysis was performed by using SPSS 16.0. Kolmogorov-Smirnov Test (K-S test) was used to explore normal distribution of the data. The K-S test results indicated that the CTx level ($Z = 1.195$, $P = 0.115$) accord with the criteria of normal distribution. χ^2 test was employed to compare the CTx level (with different level of FSH) among all of three groups. Student's t test was used for evaluation of differences between two groups. Spearman rank correlation was used in correlation analysis for FSH and CTx levels. Differences were considered statistically significant for values of $P < 0.05$.

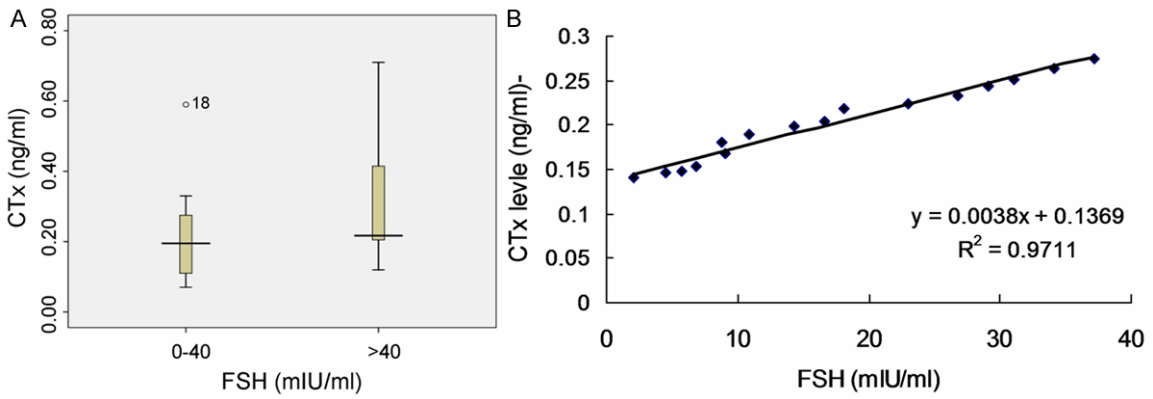

Figure 2. CTx levels in both 0-40 mIU/ml and > 40 mIU/ml FSH group, and its correlation with FSH levels of < 40 years old women. A. CTx level in both 0-40 mIU/ml and > 40 mIU/ml FSH. B. Correlation between CTx levels and FSH levels.

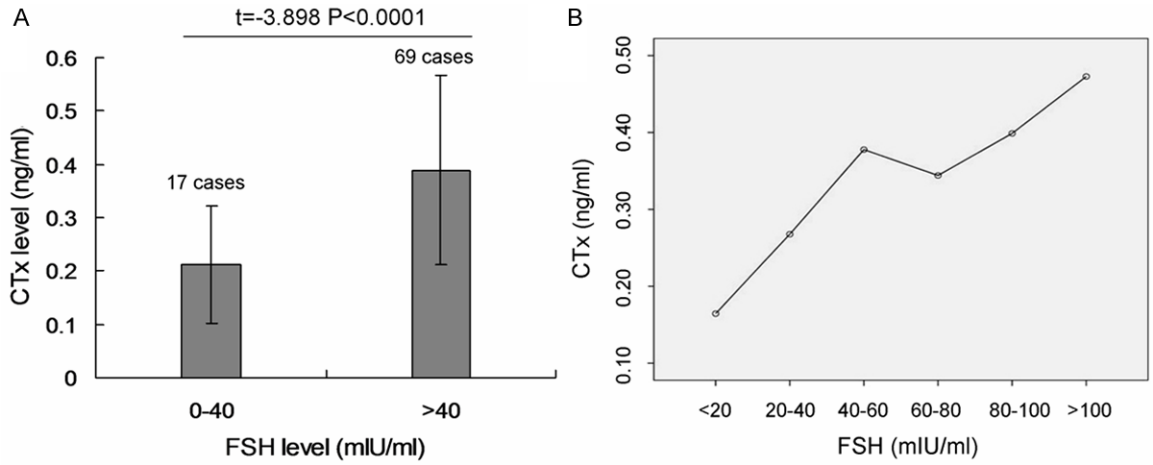

Figure 3. CTx levels in both 0-40 mIU/ml and > 40 mIU/ml FSH group, and its correlation with FSH levels of 40-50 years old women. A. CTx level in both 0-40 mIU/ml and > 40 mIU/ml FSH. B. Correlation between CTx levels and FSH levels. The different values among groups were indicated upper of the Figure.

Results

CTx levels vary differently among three groups

The result indicated that the CTx level was 0.2513 ng/ml in < 40 group, 0.3499 ng/ml in 40-50 group and 0.3545 ng/ml in 50-60 group (**Figure 1**). Therefore, the CTx levels were different among the different aged group, with the increasing level following with the increased age.

The FSH level in < 40 years women positively correlated with CTx level

There are 25 cases of women less than 40 years, the average age is 0.2513 ± 0.1640 ng/ml. Among these women, there were 16 cases with the levels of FSH 0 to 40 mIU/ml, and with the average CTx level of 0.2106 ± 0.1309 ng/ml (**Table 1**). There were 9 cases with the levels of FSH more than 40 mIU/ml, and with the average CTx level of 0.3236 ± 0.1983 ng/ml. However, there was no significant difference between the FSH level of 0 to 40 mIU/ml and more than 40 mIU/ml (**Figure 2A**; **Table 1**, t = -1.719, P = 0.099).

The correlation analysis results showed that the FSH level in < 40 years women was positively correlated with CTx level (**Figure 2B**, r = 0.8712, P < 0.05).

The FSH level in 40-50 years women positively correlated with CTx level

The average level of CTx in 40-50 years group is 0.3499 ng/ml. There were 17 cases with the

Table 2. CTx levels in different FSH level of 40-50 years old women

FSH	Case	Mean	Standard deviation	95% CI		Minimum	Maximum
				Lower	Upper		
0-40	17	0.2131	0.1101	0.1564	0.2697	0.10	0.54
40~	69	0.3893	0.1777	0.3466	0.4321	0.09	0.87
Total	86	0.3545	0.1804	0.3158	0.3932	0.09	0.87

Table 3. CTx levels in different FSH level of 50-60 years old women

FSH	Cases	Mean	Standard deviation	95% CI		Minimum	Maximum
				Lower	Upper		
0-40	9	0.3632	0.1833	0.2223	0.5042	0.14	0.64
40~	44	0.3472	0.1483	0.3021	0.3923	0.05	0.77
Total	53	0.3499	0.1529	0.3077	0.3921	0.05	0.77

FSH level rang from 0 to 40 ng/ml, and the average level of CTx was 0.2131 ± 0.1101 ng/ml. The confidential interval (CI) was range from 0.1564 to 0.2697 ng/ml. There were 69 cases with the FSH level higher than 40ng/ml, and the average level of CTx was 0.3893 ± 0.1777 ng/ml. The CI was range from 0.3446 to 0.4321 ng/ml (**Table 2**). There were significant differences for the CTx level between 0-40 group and > 40 group (**Figure 3A**; **Table 2**, $P < 0.0001$).

Also, the correlation between CTx level and FSH was analyzed. The spearman rank correlation analysis results indicated that the CTx level was positively correlated with the level of FSH in 40-50 years old group (**Figure 3B**, $P < 0.05$).

No significant correlation between FSH level in 50-60 years and CTx level

The average level of CTx in 50-60 years group is 0.3545 ng/ml. There were 9 cases with the FSH level rang from 0 to 40 ng/ml, and the average level of CTx was 0.3632 ± 0.1833 ng/ml. The confidential interval (CI) was range from 0.2223 to 0.5042 ng/ml. There were 44 cases with the FSH level higher than 40 ng/ml, and the average level of CTx was 0.3472 ± 0.1483 ng/ml. The CI was range from 0.3021 to 0.3923 ng/ml (**Table 3**). However, there were significant differences for the CTx level between 0-40 group and > 40 group in 50 to 60 years old women (**Table 3**, $P = 0.778$).

The spearman rank correlation analysis results also showed that there were no significant correlation between CTx level and FSH level in 50-60 years old women (data not show).

Discussion

When women enter the menopausal transition, the function of ovarium begins to decline, ovulation begins to stop, folliculus begin to decrease. The level of estrogen decrease gradually, and hypothesis can't receive the feedback and inhibition function of estrogen, therefore, the FSH secretion increase significantly. A study for 36 women (range from 20 to 50 years) diagnosed as osteoporosis showed that FSH could affect the formation, function and survival of osteocyte by stimulating TNF-α, IL-1, IL-6, and regulate the bone mass in a further step [9, 10]. Xu et al. [11] selected 699 health women (range from 20 to 82 years old), and monitored the serum FSH and bone turnover rate. They found that increased level of FSH could accelerate bone turnover rate, and increase the risk of bone loss and cataclasis.

Recent reports indicated that the enhance of the bone metabolism biomarkers, especially for the bone resorption biomarkers, could predict the bone fracture risk of Osteoporosis in women patients [12, 13]. Ninety percentage of the organic principle in human bone are composed of collagen protein I. The amounts, structure and stability changes of collagen I could accelerate the bone turnover, and enhance bone cells activity, and decrease the collagen entering into blood. The CTx is the catabolite in the process of collagen metabolize, and which is also the index reflecting the bone absorption [14]. When the collagen I changes significantly, the CTx level could be increased significantly in the blood [15, 16]. The previous studies proved that CTx could predict the risk of fracture with higher applicable values [17]. There are many distinguished reports about the relationship between FSH and CTx levels. Garcia-Martin et al. [18] discovered that the enhanced level of FSH could trigger the increase of CTx in serum, strengthen osteoclast activity, accelerate bone turnover, and finally cause the Osteoporosis. Our study results indicated that there are no significant differences between FSH < 40 mIU/ml and FSH > 40 mIU/ml for less than 40 years old and more than 50 years old women. However, for the 40 to 50 years old women,

when FSH > 40 mIU/ml, there is significant difference between FSH < 40 mIU/ml and FSH > 40 mIU/ml. Rannevik et al. [19] detected the endocrine changes for 12 years from menopausal transition to menopause periods. The results indicated that the FSH level was significantly enhanced at 6 years before the final menstrual period. The FSH achieved the top level at the final menstrual period, and decreased gradually in the following 10 years. In our report, the CTx level was increased in 40-50 years group, and was decreased in more than 50 years old group. Furthermore, enhanced FSH level could increase the CTx level in the 40 to 50 years group. The results also showed that there was significant change for FSH level, strength of osteoclast activity, accelerating of bone turnover in the 40 to 50 years. We speculated that the FSH level enhancement may be the main reason for bone loss in menopausal transition women. Meantime, we hold the view that this period may be the window phase for the treatment of clinical osteoporosis after menopause periods.

In summary, it's clinically significant for the combining examination of FSH and CTx in menopausal transition women, which could observe the bone metabolize changes quickly and sensitively, and prevent or therapy the osteoporosis in a further step. Furthermore, examination of the FSH and CTx could also identify the effects of drug therapy on bone metabolize, which could be useful to regulate the therapeutic strategy in a short time. However, with the limit of sample amounts and source, the conclusion of this study may only applicable for women in Zhejiang Province. We would amplify the range of research, improve the experiment method, and follow-up the above population in the further study.

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Disclosure of conflict of interest

None.

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