Relationship between Serum Ferritin Levels and Biochemical Markers of Bone Turnover in Postmenopausal Women

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Abstract

Objective: To study the relationship between serum ferritin levels and biochemical markers of bone turnover in postmenopausal women.

Methods: This cross-sectional study was conducted at the Department of Obstetrics and Gynecology, Faculty of Medicine Vajira Hospital, Bangkok, Thailand. One hundred and twenty four postmenopausal Thai women were recruited from menopause clinic between December 2015 and March 2016. The inclusion criteria were age 40 years or more, body mass index (BMI) of 19-30 kg/m^2, not use menopausal hormone therapy (MHT) within the last 3 months, adequate cessation period of any bone antiresorptive agent, no underlying disease which possibly associated with chronic anemia, and no history of steroid hormone, anabolic agent, or anticoagulant use. The subjects were excluded if their blood specimens were unsuccessfully collected or incompletely analyzed. Two kinds of biochemical markers of bone turnover were selected to study. The first one was a bone-formation marker, called procollagen type I N- terminal propeptide (P1NP) and the second was a bone-resorption marker, namely C-terminal cross-linked telopeptide of type I collagen (CTx). Each marker was evaluated its relationship to serum ferritin.

Results: After recruitment, one subject was excluded because her blood specimen was loss during transfer, therefore 123 cases were left for analysis. Mean (SD) age, BMI, duration of menopause, and number of parity of the overall subjects were 56.8 (4.2) years, 24.3 (3.1) kg/m^2, 7.1 (5.3) years, and 1.3 (1.1), respectively. Mean (SD) serum ferritin, P1NP, and CTx levels were 147.75 (95.11), 63.90 (16.70), and 0.463 (0.154) ng/ml, respectively. Serum ferritin levels were negatively correlated with P1NP and with CTx levels (r = -0.149, p = 0.099 and r = -0.038, p = 0.677, respectively).

Conclusion: Serum ferritin levels had non-significant inverse relationship with P1NP and CTx levels in postmenopausal women. The long-term effects of low iron storage on biochemical markers of bone turnover are needed to be further evaluated.

Keywords: Iron deficiency anemia, ferritin, osteoporosis, biochemical markers of bone turnover

ความสัมพันธ์ระหว่างระดับเฟอร์ริตินในน้ำเหลืองของเลือดและดัชนีชีวเคมีของกระบวนการสร้างและสลายกระดูกในสตรีวัยหมดระดู

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บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาความสัมพันธ์ระหว่างระดับเฟอร์ริตินในน้ำเหลืองของเลือดและดัชนีชีวเคมีของกระบวนการสร้างและสลายกระดูกในสตรีวัยหมดระดู

วิธีดำเนินการวิจัย: ดำเนินการศึกษาแบบภาคตัดขวาง ณ ภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์วชิรพยาบาล มหาวิทยาลัยนวมินทราธิราช กรุงเทพมหานคร ประเทศไทย โดยคัดเลือกสตรีไทยวัยหมดระดูจำนวน 124 คน ที่มารับการตรวจในคลินิกวัยทอง ตั้งแต่เดือนธันวาคม พ.ศ. 2558 ถึง เดือนมีนาคม พ.ศ. 2559 โดยมีเกณฑ์คัดเข้า ได้แก่ อายุ 40 ปีขึ้นไป มีค่าดัชนีมวลกาย 19-30 กิโลกรัม/เมตร^2 ไม่มีการใช้ฮอร์โมนเพื่อรักษาภาวะหมดระดูใน 3 เดือนที่ผ่านมา หยุดยาต้านการสลายกระดูกมาในระยะเวลาที่เพียงพอ ไม่มีโรคประจำตัวที่สัมพันธ์กับภาวะโลหิตจางเรื้อรัง และไม่มีประวัติการใช้สารสเตียรอยด์ สารอะนาบอลิก หรือยาละลายลิ่มเลือด อาสาสมัครจะถูกคัดออกในกรณีที่เก็บตัวอย่างเลือดไม่ได้หรือมีผลการวิเคราะห์ที่ไม่สมบูรณ์ ดัชนีชีวเคมีที่ทำการศึกษามี 2 ชนิด ได้แก่ ดัชนีการสร้างกระดูกชนิด procollagen type I N- terminal propeptide (P1NP) และดัชนีการสลายกระดูกชนิด C-terminal cross-linked telopeptide of type I collagen (CTx) ค่าของดัชนีแต่ละชนิดจะนำไปวิเคราะห์หาความสัมพันธ์กับระดับเฟอร์ริตินในน้ำเหลืองของเลือด

ผลการวิจัย: หลังการคัดเข้า มีอาสาสมัคร 1 รายถูกคัดออกเนื่องจากตัวอย่างเลือดที่เก็บมามีการสูญหายระหว่างนำส่งตรวจ จึงเหลืออาสาสมัครที่จะนำมาวิเคราะห์จำนวน 123 ราย ค่าเฉลี่ย (ส่วนเบี่ยงเบนมาตรฐาน) อายุ ดัชนีมวลกาย ระยะเวลาการหมดระดู และจำนวนการคลอดของอาสาสมัครทั้งหมด เท่ากับ 56.8 (4.2) ปี 24.3 (3.1) กิโลกรัม/เมตร^2 7.1 (5.3) ปี และ 1.3 (1.1) ตามลำดับ ค่าเฉลี่ย (ส่วนเบี่ยงเบนมาตรฐาน) ของระดับเฟอร์ริตินในน้ำเหลืองของเลือด P1NP และ CTx เท่ากับ 147.75 (95.11), 63.90 (16.70), และ 0.463 (0.154) นาโนกรัม/มิลลิลิตร ตามลำดับ ระดับเฟอร์ริตินในน้ำเหลืองของเลือดมีความสัมพันธ์ในทิศทางตรงกันข้ามกับระดับของ P1NP และ CTx (r = -0.149, p = 0.099 and r = -0.038, p = 0.677 ตามลำดับ)

สรุป: ระดับเฟอร์ริตินในน้ำเหลืองของเลือดในสตรีวัยหมดระดู มีความสัมพันธ์ในทิศทางตรงกันข้ามกับระดับของ P1NP และ CTx อย่างไม่มีนัยสำคัญ จำเป็นต้องทำการศึกษาเพิ่มเติมต่อไปเกี่ยวกับผลระยะยาวของการมีธาตุเหล็กสะสมในปริมาณต่ำ ที่มีต่อดัชนีชีวเคมีของกระบวนการสร้างและสลายกระดูก

คำสำคัญ: ภาวะโลหิตจางจากการขาดธาตุเหล็ก, เฟอร์ริติน, โรคกระดูกพรุน, ดัชนีชีวเคมีของกระบวนการสร้างและสลายกระดูก

Introduction

Osteoporosis is a common health problem among postmenopausal women[1]. It has long been believed that pathophysiology of the disease in women of this age-group is related to estrogen deficiency after menopause[2]. However, much previous epidemiological data showed that osteoporosis was not present in every postmenopausal women[3]. Such information suggested that mechanisms of this disease are possibly complex[4]. Even estrogen deficiency might be a main contributor it is not the sole risk factor of the development of postmenopausal osteoporosis. Several known risk factors which participate in the mechanisms are age, genetics, inadequate calcium and vitamin D intake, smoking and thinness[5]. At present, there is still no conclusion about which factor is the most important, therefore the diagnosis of osteoporosis is based primarily on screening tools developed by combining multiple risk factors[5].

Because prior researches reported that osteoporosis was 2 to 6 times more prevalent in female than male population[1,6], thereafter later studies usually focused on risk factors of the disease that specific to female sex, for example, serum estrogen levels. However, the results did not show any statistically significant association between serum estrogen levels and bone turnover rates[5].

Other health factors that probably relate to osteoporosis in female population are iron deficiency anemia (IDA)[7-10] and hemoglobin levels[11-12]. Previous study on hematologic status revealed that IDA was more common and average hemoglobin levels were less in women than men[13]. In other words, women usually have lower iron storage for hemoglobin synthesis because of repeated losses during menstruations and pregnancies[14].

Continuing iron losses during pubertal and reproductive ages may induce bone marrow to be more active in women than men. Bone marrow hyperactivity in women, on one hand, is a physiologic compensation resulting in a more red blood cell progenitor synthesis for the prevention of IDA[4]. On the other hand, this hyperactivity is supposed to be a pathophysiologic process which cause an excessive proliferation of other progenitor cells including osteoclasts[4]. Excessive osteoclast proliferation may induce a faster bone resorption and a more prevalent osteoporosis in women than men[4].

Owing to the evidence that depleted iron storage may affect bone turnover rate especially in women[4], there were several studies those evaluating the associations between IDA and osteoporosis[7-9,12]. All of those studies used hemoglobin and/or hematocrit levels as markers of IDA and Bone Mineral Density (BMD) or the incidence of osteoporotic fracture as a marker of osteoporosis. There was only one study which analyzed relationship between markers of iron storage e.g. serum ferritin and biochemical markers of bone turnover[10]. That study showed a significant reverse relationship between serum ferritin and bone resorption marker. However, the study was conducted in premenopausal women. Because of the shortness of study which designed to investigate the relationship between markers of iron storage and biochemical markers of bone turnover in postmenopausal period, the present study was designed to evaluate the relationship between serum ferritin levels and biochemical markers of bone turnover in postmenopausal women.

Methods

This cross-sectional study was conducted at the Department of Obstetrics and Gynecology, Faculty of Medicine Vajira Hospital, Bangkok, Thailand. One hundred and twenty four postmenopausal Thai women, aged 40 years or more, were recruited from the menopause clinic between December 2015 and March 2016. Subjects were included to study if they had body mass index (BMI) of 19-30 kg/m^2, no prior use of menopausal hormone therapy (MHT) within the last 3 months,

and adequate cessation period after osteoporosis treatment with antiresorptive agents, i.e. at least 2 years after intravenous regimen, 1 year after subcutaneous regimen, and 2 years, 1 year, 6 months, and 2 months after oral regimens for 48 weeks or longer, 8-48 weeks, 2-8 weeks, and less than 2 weeks, respectively. All studied subjects had no history of gastrointestinal bleeding, malabsorption, gastric ulcers, gastrointestinal tract surgeries, inflammatory bowel diseases, coronary artery diseases or congestive heart failure, chronic renal failure, thyroid diseases, parathyroid diseases, malignancies, hematologic diseases, or vitamin D deficiency, and no history of steroid hormone, anabolic agent, or anticoagulant use. Informed consent process were performed by investigator or the nurse at menopause clinic who was assigned as a research assistant in this study. Subjects were excluded if they withdrew from the study, refused to undergo venous puncture or their blood samples were unsuccessfully or inadequately collected. Subjects were also excluded if their blood specimens were not completely analyzed due to any error on the handling or transferring process. Additional BMD tests were performed in subjects who had the levels of biochemical markers of bone turnover higher than the cut-off points (48.35 ng/ml for P1NP or 0.328 ng/ml for CTx). Then standard treatments of osteoporosis were given if they had T-score of BMD less than -2.5. Subjects who had ferritin levels below the cut-off point of iron deficiency (100.00 ng/ml) were referred to hematologist for further investigations and treatments.

The sample size was estimated by using Pearson's correlation coefficient between ferritin level and biochemical marker of bone turnover, named as procollagen N-terminal propeptide from the previous study which revealed the value of 0.73[15]. Initially, with a level of bilateral significance of 95% (α error=0.05) and a power of 80% (β error=0.2), the sample size was 13 subjects. Then the number was increased to be 113 because the relationships between 10 other studied factors and biochemical markers of bone turnover were also planned to be analyzed. Finally, the recruitment was increased up to 124 for preventing a probable 10% loss of the data.

The study was approved by the Institutional Review Board of the Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand, and all subjects provided their written informed consent before recruitment.

Two kinds of biochemical markers of bone turnover were selected to study. The first one was a bone-formation marker, called procollagen type I N- terminal propeptide (P1NP) and the second was a bone-resorption marker, namely C-terminal cross-linked telopeptide of type I collagen (CTx). Each marker was evaluated its relationship to serum ferritin. After recruitment, subjects were undergone history taking and physical examinations to collect and record for baseline data and controlled factors including age, body built (weight, height and BMI), duration of menopause, parity, history of MHT use, underlying diseases, history of osteoporosis/ fractures, history of cigarette smoking/ alcohol consumption, levels of education and physical activity levels (PAL). Then, each subject's blood sample was drawn from cubital vein in a volume of 6-10 milliliters on the next official day between 8:00 and 9:00 a.m. after an overnight fast for at least 8 hours. Blood samples were collected in EDTA blood tubes and then sent to be analyzed for the serum levels of ferritin, P1NP and CTx.

Laboratory assays

Blood samples were centrifuged at 3,000 rpm for 10 min and the separated serum samples were stored at -20°C until assay. Ferritin, P1NP and CTx levels were analyzed by a commercially available electrochemiluminescence immunoassay or ECLIA (Roche Elecsys 2010, Mannheim, Germany). The lower-upper detection limits of the assay are 0.5-2,000, 5-1,200 and 0.01-6 ng/ml for ferritin,

P1NP, and CTx, respectively. And the coefficients of variation are ranged from 1.5-2.5, 1.2-2.1, and 2.2-3.5% for ferritin, P1NP, and CTx, respectively. Normal ranges of ferritin, P1NP, and CTx levels are 13.00-150.00, 40.78-48.35, and 0.293-0.328, respectively. Laboratory assays in this study were performed in-house, and calibrated in every new lot of reagents. Internal and external quality controls were tested in daily and monthly basis, respectively.

Statistical analysis

Data presentation and statistical analysis were performed as following. Descriptive statistics (mean (standard deviation, SD) or median (range) and percentage were used to express demographic, baseline, and measurement outcome data. Relationships between each biochemical marker of bone turnover (P1NP and CTx) and ferritin were tested by an analysis of Pearson's correlation. All other controlled factors which affecting bone turnover were analyzed by chi-square or Fisher-exact test. A p level of < 0.05 was considered statistically significant.

Results

One hundred and twenty four subjects who met the inclusion criteria were recruited to the study. One subject was excluded because her blood specimen was loss during transfer, therefore 123 cases were left for analysis. The mean (SD) age, BMI, duration of menopause, and number of parity of the overall subjects were 56.8 (4.2) years, 24.3 (3.1) kg/m^2, 7.1 (5.3) years, and 1.3 (1.1), respectively. The overall mean (SD) ferritin, P1NP, and CTx levels were 147.75 (95.11), 63.90 (16.70), and 0.463 (0.154) ng/ml, respectively. Serum ferritin levels were negatively correlated with P1NP and with CTx levels but the degree of correlations were not statistically significant (r = -0.149, p = 0.099 and r = -0.038, p = 0.677, respectively). Both correlations are shown in figure 1 and 2.

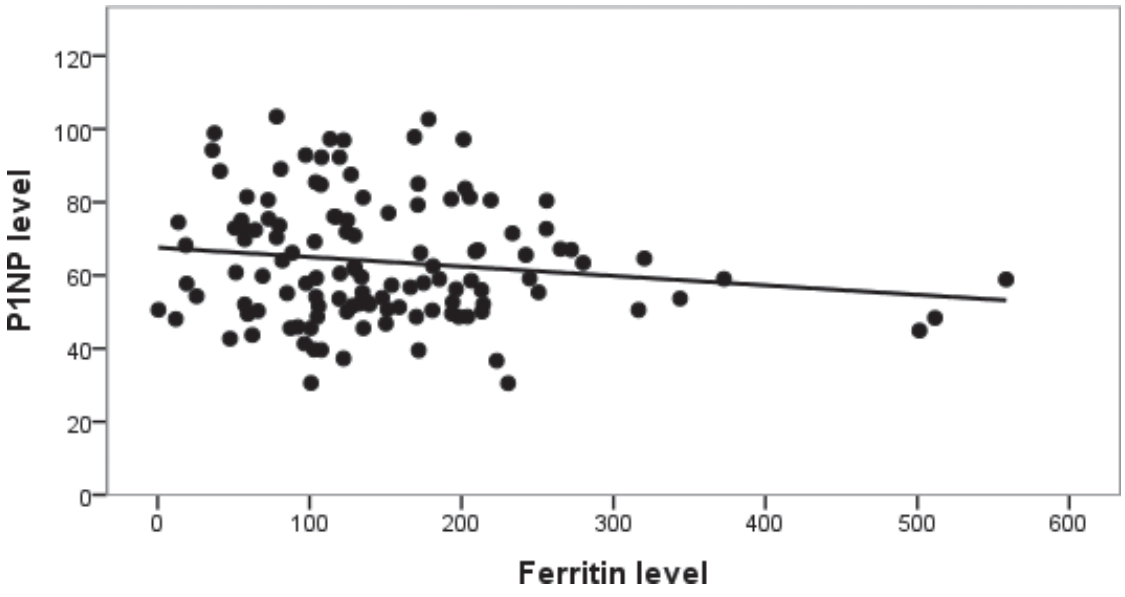

Figure 1: Pearson's correlation between ferritin and procollagen type I N- terminal propeptide (P1NP) levels

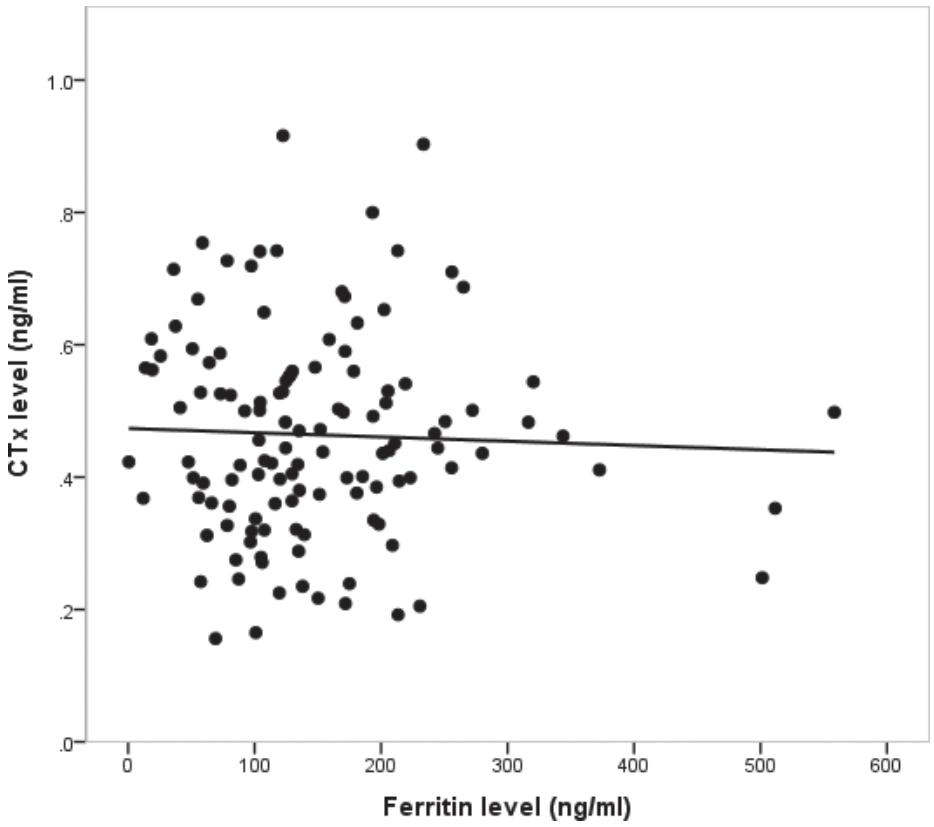

Figure 2: Pearson's correlation between ferritin and C-terminal cross-linked telopeptide of type I collagen (CTx) levels

When categorized subjects by the levels of biochemical markers of bone turnover, there was no statistically significant difference in subjects' characteristics between groups of normal and high P1NP and CTx levels (Table 1).

A proportion of subjects who had history of prior use of MHT is statically higher in normal CTx group than high CTx group (20.83% vs. 3.03%, p = 0.007). Subjects in high P1NP group had statistically significant higher PAL than normal P1NP group (63.21% vs 29.41% who had moderate to high activity, p = 0.024). All other studied factors categorized by levels of biochemical markers of bone turnover are shown in Table 2.

Discussion

Relationship between IDA and osteoporosis has been hypothesized and frequently studied since the last 10 years[7-10,12]. However, results of those studies are still conflicting and most of the studies used hemoglobin and/or hematocrit levels as markers of IDA and BMD or the incidence of osteoporotic fracture as a marker of osteoporosis[7-9,12]. Serum ferritin is the best international standard and the least invasive single test for the evaluation of iron storage[17]. Additionally, biochemical markers of bone turnover were claimed in many previous reports that they were beneficial for clinical uses in terms of predicting the risk of occurrence and monitoring the therapeutic response of osteoporosis[18-23]. However, there was only one

study which analyzed relationship between serum ferritin and biochemical markers of bone turnover[10]. Therefore, such a relationship is valuable to be additionally studied and, in my knowledge, this study might be one of the few which design to investigate this relationship.

Both results of correlation analysis between ferritin and P1NP, and, ferritin and CTx in this study were in negative direction. These seem to answer the hypothesis that the lower iron storage might cause the higher bone marrow activity resulting in more proliferation of progenitor cells including osteoclasts which activate bone remodeling. Nevertheless, the degree of both correlations was low and did not reach statistical significance. Thus, whether IDA is the risk factor of osteoporosis could not be concluded. Design of this study that was cross-sectional may involve in the non-significant results. Because it is possible that the status of low iron storage might need to sustain for a longer period before inducing an increase in bone remodeling activity. Therefore, studies which are prospectively designed might be more appropriate to prove this assumption.

When compared with the previous one study which also analyzed relationship between markers of iron storage and biochemical markers of bone turnover[10], this study had both the different and the same results. The previous study showed that IDA subjects had statistically significant higher bone-resorption marker than non-IDA subjects, but this study did not. On contrary, both studies also showed that IDA did not have statistically significant effect on the levels of bone-formation marker. However, the previous study conducted in premenopausal women with continuing menstrual cycles. IDA in the studied group might have a more severity and/or longer duration than in this study, therefore effects on the change of bone-resorption marker were more obvious.

Table 1:

Characteristic factors of subjects categorized by levels of biochemical markers of bone turnover. Data are given as mean (SD).

Factors	Markers levels					
	P1NP* (ng/ml)		*P*	CTx* (ng/ml)		*p*
	Normal (n=17)	High (n=106)		Normal (n=24)	High (n=99)	
Age (years)	57.71 (4.65)	56.68 (4.10)	0.348[a]	55.79 (3.80)	57.07 (4.24)	0.179[a]
BMI (kg/m^2)	23.77 (3.16)	24.35 (3.15)	0.482[a]	24.95 (2.98)	24.11 (3.17)	0.238[a]
Duration of menopause (years)	7.12 (5.61)	7.05 (5.29)	0.959[a]	7.46 (5.18)	6.96 (5.36)	0.681[a]
Ferritin levels (ng/ml)	103.40 (109.12)	149.32 (93.15)	0.650[a]	140.394 (90.39)	149.53 (96.57)	0.675[a]

BMI; body mass index, P1NP; procollagen type I N- terminal propeptide, CTx; C-terminal cross-linked telopeptide of type I collagen,
* Cut-off points between normal and high levels for P1NP and CTx are 48.35 and 0.328 ng/ml, respectively. a; t-test

Table 2:

Proportion of subjects categorized by studied factors and levels of biochemical markers of bone turnover. Data are given as number (percent).

Factors	Markers levels					
	P1NP* (ng/ml)		*p*	CTx* (ng/ml)		*p*
	Normal (n=17)	High (n=106)		Normal (n=24)	High (n=99)	
Ferritin level** (ng/ml)						
Normal	11 (64.71)	75 (70.75)	0.255[a]	16 (66.67)	70 (70.71)	0.150[a]
Low	6 (35.29)	31 (29.25)		8 (33.33)	29 (29.29)	
MHT use						
Never	16 (94.12)	99 (93.40)	1.000[b]	19 (79.17)	96 (96.97)	0.007[b]
Prior use	1 (5.88)	7 (6.60)		5 (20.83)	3 (3.03)	
Underlying diseases***						
Absent	15 (88.24)	100 (94.34)	0.305[b]	21 (87.50)	94 (94.95)	0.187[b]
Present	2 (11.76)	6 (5.66)		3 (12.50)	5 (5.05)	
Previous osteoporosis/ fractures	16 (94.12)	103 (97.17)	0.453[b]	23 (95.83)	96 (96.97)	1.000[b]
No	1 (5.88)	3 (2.83)		1 (4.17)	3 (3.03)	
Yes						
Physical activity levels						
Sedentary	4 (23.53)	6 (5.66)	0.024[b]	2 (8.33)	8 (8.08)	0.655[b]
Low	8 (47.06)	33 (31.13)		9 (37.50)	32 (32.32)	
Moderate	5 (29.41)	59 (55.66)		13 (54.17)	51 (51.52)	
High	0 (0.00)	8 (7.55)		0 (0.00)	8 (8.08)	

MHT; menopausal hormone therapy, P1NP; procollagen type I N- terminal propeptide, CTx; C-terminal cross-linked telopeptide of type I collagen, * Cut-off points between normal and high levels for P1NP and CTx are 48.35 and 0.328 ng/ml, respectively. ** Cut-off point between normal and low levels ferritin is 100.00 ng/ml[16]. ***diabetes mellitus/ hypertension/ dyslipidemia, a; chi-square test, b; Fisher exact test

Two of all other studied factors which were statistically significant associated with the changes in biochemical markers of bone turnover were history of prior use of MHT and PAL. The prior use of MHT was present in a higher proportion in normal CTx subjects. Long-term positive effect of MHT on bone health i.e. decreased bone resorption was confirmed in the study which start the therapy in early postmenopausal period[18]. Moderate to high PAL was found to be associated with high P1NP level in this study. Such an association may reflect that higher physical activity can stimulate more bone formation and could support the theory of life style risk factors of osteoporosis. However, the sample size of this study was not calculated based on the association of these two factors with biochemical markers of bone turnover, so the results should be interpreted with caution. In addition, these results are not new knowledge and also expected to be found.

This study was limited by non-prospective design, no randomization between the studied and controlled groups, and small number of subjects.

In conclusion, serum ferritin levels had non-significant inverse relationship with P1NP and CTx levels in postmenopausal women. Laboratory investigations to determine iron storage are still not recommended to use as screening tests of osteoporosis in postmenopausal women. The long-term effects of low iron storage, categorized by different duration and/or severity, on biochemical markers of bone turnover are needed to be further evaluated.

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References

1. Limpaphayom KK, Taechakraichana N, Jaisamrarn U, Bunyavejchevin S, Chaikittisilpa S, Poshyachinda M, et al. Prevalence of osteopenia and osteoporosis in Thai women. Menopause 2001; 8: 65-9.
2. Tella SH, Gallagher JC. Prevention and treatment of postmenopausal osteoporosis. J Steroid Biochem Mol Biol 2014; 142: 155-70.
3. Gómez-de-Tejada Romero MJ, Navarro Rodríguez MD, Saavedra Santana P, Quesada Gómez JM, Jódar Gimeno E, Sosa Henríquez M. Prevalence of osteoporosis, vertebral fractures and hypovitaminosis D in postmenopausal women living in a rural environment. Maturitas 2014; 77: 282-6.
4. Gurevitch O, Slavin S. The hematological etiology of osteoporosis. Med Hypotheses 2006; 67: 729-35.
5. North American Menopause Society. Management of osteoporosis in postmenopausal women: 2010 position statement of The North American Menopause Society. Menopause 2010; 17: 25-54.
6. Pongchaiyakul C, Apinyanurag C, Soontrapa S, Soontrapa S, Pongchaiyakul C, Nguyen TV, et al. Prevalence of osteoporosis in Thai men. J Med Assoc Thai 2006; 89: 160-9.
7. D'Amelio P, Cristofaro MA, Tamone C, Morra E, Di Bella S, Isaia G, et al. Role of iron metabolism and oxidative damage in postmenopausal bone loss. Bone 2008; 43: 1010-5.
8. Chen Z, Thomson CA, Aickin M, Nicholas S, Van Wyck D, Lewis CE, et al. The relationship between incidence of fractures and anemia in older multiethnic women. J Am Geriatr Soc 2010; 58: 2337-44.
9. Korkmaz U, Korkmaz N, Yazici S, Erkan M, Baki AE, Yazici M, et al. Anemia as a risk factor for low bone mineral density in postmenopausal Turkish women. Eur J Intern Med 2012; 23: 154-8.
10. Wright I, Blanco-Rojo R, Fernández MC, Toxqui L, Moreno G, Pérez-Granados AM, et al. Bone remodelling is reduced by recovery from iron-deficiency anaemia in premenopausal women. J Physiol Biochem 2013; 69: 889-96.
11. Cesari M, Pahor M, Lauretani F, Penninx BW, Bartali B, Russo R, et al. Bone density and hemoglobin levels in older persons: results from the InCHIANTI study. Osteoporos Int 2005; 16: 691-9.
12. Laudisio A, Marzetti E, Pagano F, Bernabei R, Zuccalà G. Haemoglobin levels are associated with bone mineral density in the elderly: a population-based study. Clin Rheumatol 2009; 28: 145-51.
13. Salvin HE, Pasricha SR, Marks DC, Speedy J. Iron deficiency in blood donors: a national cross-sectional study. Transfusion 2014; 54: 2434-44.
14. Clark SF. Iron deficiency anemia. Nutr Clin Pract 2008; 23: 128-41.
15. Jensen PD, Heickendorff L, Helweg-Larsen HM, Jensen FT, Christensen T, Ellegaard J. Serum procollagen III peptide concentration in iron overload. Eur J Haematol 1996; 57: 157-64.

16. Anker SD, Colet JC, Filippatos G, Willenheimer R, Dickstein K, Drexler H, et al. Ferric carboxymaltose in patients with heart failure and iron deficiency. N Engl J Med 2009; 361: 2436-48.
17. Polina V, Coriata R, Perkinsa G, Dhoogea M, Abitbola V, Leblanc S, et al. Iron deficiency: from diagnosis to treatment. Dig Liver Dis 2013; 45: 803-9.
18. Bjarnason NH, Christiansen C. Early response in biochemical markers predict long-term response in bone mass during hormone replacement therapy in early postmenopausal women. Bone 2000; 26: 561-9.
19. Dogan E, Posaci C. Monitoring hormone replacement therapy by biochemical markers of bone metabolism in menopausal women. Postgrad Med J 2002; 78: 727-31.
20. Seibel MJ. Clinical application of biochemical markers of bone turnover. Arq Bras Endocrinol Metabol 2006; 50: 603-20.
21. Lewiecki EM. Prevention and treatment of postmenopausal osteoporosis. Obstet Gynecol Clin N Am 2008; 35: 301-15.
22. Vasikaran SD. Utility of biochemical markers of bone turnover and bone mineral density in management of osteoporosis. Crit Rev Clin Lab Sci 2008; 45: 221-58.
23. Garnero P. Bone markers in osteoporosis. Curr Osteoporos Rep 2009; 7: 84-90.