Association Between Serum Ferritin Levels and Low Bone Mineral Density in Postmenopausal Osteoporosis Women

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Abstract- As women go through menopause, serum estrogen decreases, and ferritin increases. Ferritin is an essential component of the body, but many studies have stated that ferritin, which exceeds the normal physiological range, may potentially cause health problems in women. The aim of this study is to investigate the relationship between bone mineral density and serum ferritin levels in post-menopausal women and to evaluate serum ferritin levels as a potential biomarker for postmenopausal osteoporosis. Serum ferritin levels were measured in 62 postmenopausal women with low bone mineral density, and in 18 postmenopausal healthy control women using a standardized Enzyme-Linked Immune Sorbent Assay (ELISA) kit. Bone mineral density BMD was assessed at the lumbar spine and femoral neck. The mean serum ferritin level was significantly higher in the postmenopausal women with low BMD group (group 1) than in the normal control group (group 2), respectively (mean=262.69 vs. 181.44 ng/ml, (P<0.05), and serum ferritin level was negatively correlated with BMD among low BMD postmenopausal women's group (R= -0.628, P=0.0001), and in the healthy postmenopausal group (R= -0.052, P=0.838). A comparison of the BMD between spine and femur neck sites shows that the frequency of low BMD in the spine site is higher than the femur neck site. Our findings show that increased serum ferritin levels were associated with low bone mineral density in postmenopausal osteoporosis.

Introduction

Osteoporosis is the most common metabolic bone disorder and remains an increasingly significant problem, affecting 200 million individuals worldwide (1). Osteoporosis is characterized by low bone mass, structural deterioration, and porous bone, which are associated with higher fracture risk (2).

Bone is a dynamic tissue that constantly undergoes a turnover in order to maintain stability and integrity. In this process called bone turnover or bone remodeling, two effector cell types are involved. Osteoclasts specialized for bone resorption, and osteoblasts, responsible for bone formation, are key players in bone turnover (3).

Bone remodeling is a dynamic process required for the maintenance of bone architecture in response to the changing mechanical needs. It is also a vital process during the repair of bone tissue following injury (4).

Although osteoporosis (low bone mass) can occur at any age and in both sexes, it is more common in women than men.). In postmenopausal women, fractures due to osteoporosis are more common than stroke, myocardial infarction, and breast cancer combined. 6. For women aged 50, the lifetime risk of a fracture due to osteoporosis is 50%. A fracture can be a life-changing event and may represent a significant threat to personal independence (5).

Postmenopausal osteoporosis (PMOP) was a systemic bone metabolism disease, characterized by progressive bone loss following menopause. Estrogen deficiency as a result of menopause was known to increase bone resorption and accelerate bone loss. Previous studies have provided evidence that iron affected the bone mass only in the absence of estrogen, and the inhibition of estrogen on iron-induced osteopenia was particularly relevant to bone resorption rather than bone formation. At present, iron overload is an important risk factor for PMOP (6).

Iron was an essential trace element involved in

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human physiological functions (6). Iron is essential in oxygen transport and participates in many enzymatic systems in the body, with important roles in collagen synthesis and vitamin D metabolism (7).

However, iron overload in the body had a toxic effect on osteoblasts, gave rise to osteoporosis by inhibiting osteoblast proliferation and differentiation, and increased osteoclastogenesis (6).

Ferritin, an iron storage protein, is the primary iron storage mechanism and is critical to iron homeostasis. Ferritin makes iron available for critical cellular processes while protecting lipids, DNA, and proteins from the potentially toxic effects of iron. Alterations in ferritin are commonly seen in clinical practice, often reflecting perturbations in iron homeostasis or metabolism. It is increasingly recognized that ferritin also plays a role in a multitude of other conditions, including inflammatory, neurodegenerative, and malignant diseases (8).

Ferritin serves as a critical component of iron homeostasis. Its' primary role is in iron sequestration in which it functions as a ferroxidase, converting Fe(II) to Fe(III) as iron is internalized and sequestered in the ferritin mineral core (8).

In the present study, we compared serum ferritin levels between osteoporotic women and healthy control women, and we also investigated the relationship between serum ferritin levels with bone mineral density in postmenopausal women.

Materials and Methods

Study subjects

This prospective cross-sectional study conducted on 80 postmenopausal women divided into two groups. Group 1, had 62 postmenopausal women with reduced bone mineral density BMD, group 2 had 18 healthy control postmenopausal women with normal BMD at all of the lumbar spine and femoral neck. Subjects with chronic liver diseases, chronic renal diseases, malignancy, thyroid diseases, parathyroid diseases, iron hemostasis disease were excluded from this study. Subjects were also excluded if they had taken drugs for thyroid dysfunction and bone metabolisms, such as bisphosphonate, estrogen, glucocorticoids, and others.

All of these samples were collected between January 2016 and August 2016 at Damascus Hospital. The study was approved by the Ethical Commission of Damascus University, and written informed consent was obtained from all patients when they were enrolled.

Sampling

Whole blood was centrifuged at 3000 rpm (1509×g) for 20 min, and aliquots were stored in light-protected conditions at -80° C.

Variable measurements
Measurement of serum ferritin level

Serum ferritin levels were measured by the ELISA method using a commercial kit (DiaMetra, Italy) according to the manufacturer's instructions and spectro-photo microplate reader at Damascus University (Elysisuno - Human, Germany). Also, the serum levels of iron, total iron-binding capacity (TIBC), ALT, and AST were measured by using a Hitachi automatic analyzer 7600.

Bone mineral density measurement

Bone mineral density (BMD) was measured in the lumbar spine (L2-4) and femur neck, by using Dual Energy X-ray Absorptiometry (DEXA).

Statistical analysis

First, the distribution of serum ferritin level was determined according to the natural curve using the Kolmogorov-Smirnov test, and then the difference was evaluated in serum ferritin level between the two different groups.

Inferential analyses were done for quantitative variables using independent t-test in cases of two independent groups with parametric data.

The dependent variable was BMD measured at the lumbar spine and femur neck.

The baseline characteristics of the two groups were compared using a one-way analysis of variance (ANOVA) for continuous variables or *chi*-square tests for categorical variables.

The Correlation between serum levels of ferritin and BMD in each group was analyzed using Pearson's correlation coefficient.

Wilcoxon test was done to compare the frequency of low BMD in the spine and femur neck sites.

Statistical analyses were conducted using IBM SPSS Statistics 20 and Microsoft Excel 2010.

The results were considered statistically significant when the P was < 0.05.

Results

Characteristics of the subject

Table 1. Shows baseline characteristics of two groups of postmenopausal women in our study

Variables	Healthy postmenopausal women (group 1) (N=18)	postmenopausal women with low BMD (group 2) (N=62)
Age	52±5.21	55.5±6.30
BMI	25.44±2.99	28.96±3.28
Serum ferritin	181.44±71.23	262.69±96.52
Spine BMD	0.139±0.920	-2.297±1.031
Femur neck BMD	0.106±0.515	-1.373±0.688
Iron	70.11±20.29	74.29±25.11
TIBC	262.67±13.07	257.50±21.61
ALT	26.30±10.77	28.10±15.46
AST	25.19± 9.55	27.30±11.52

Serum ferritin was statistically significantly higher in postmenopausal women in group 2 than in group1, whereas BMD on femur neck and lumbar spine and TIBC were lower in postmenopausal women group 2 than the women in group 1. No significant differences were observed with respect to their biological parameters, iron, BMI, ALT, AST.

Table 2. Results of Pearson's correlation coefficient between BMD and ferritin in two groups of study

Second variable	Study group	First variable= BMD according to T score		
		R-value	Numbers	*P*
Serum Ferritin	postmenopausal women with low BMD	-0.628	62	0.000
Serum Ferritin	Healthy postmenopausal women	-0.052	18	0.838

Correlation between serum ferritin level and bone mineral density

The results show that the p-value is less than 0.05 in the osteoporotic patients' group, *i.e.*, at the 95% confidence level, there is a statistically significant correlation between the BMD and the serum ferritin level in this group. Since the algebraic indication of the corresponding correlation coefficient was negative, we conclude that the corresponding relations were inverse.

For the healthy group, the p-value is higher than 0.05, *i.e.,* at a 95% confidence level, there was no statistically significant linear correlation between the BMD and serum ferritin levels in the healthy group.
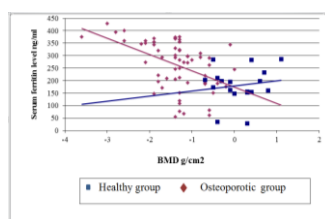

Figure 1. Association between serum ferritin and BMD among postmenopausal women in two groups of study by Pearson's correlation coefficient

Correlation between serum ferritin level and the age of women in this study

One way ANOVA test was performed to evaluate the associations between the age of women and serum ferritin levels; the results show that among all participant women, the higher serum ferritin level was observed in women with age≥ 60.
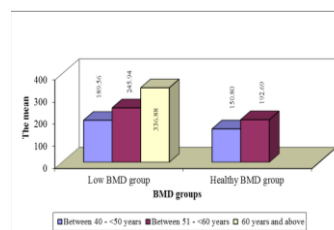
Figure 2. The mean serum ferritin level in two groups of study, according to the age groups of postmenopausal women.
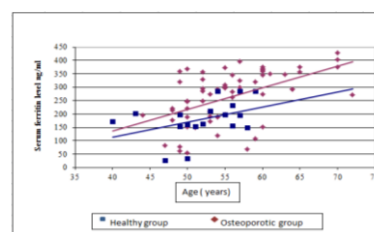
Figure 3. The prevalence of serum ferritin level (ng/ml) according to the women's age (years) in this study.

Comparison of BMD in Lumber spine and femur neck in women of this study

The T-test of the interrelated samples was performed to study the significance of differences between the mean of spine BMD and the mean femur neck BMD of the study sample as follows:

Table 3. The results of *t*-test between mean spine BMD and mean femur neck BMD of all women in the study

Comparison of BMD values depending on T score between two sites	Relative difference	t-value	Degree of freedom	*P*
Lumber spine-femur neck	-0.71	-6.506	79	0.0001

Since the *P*-value is less than 0.05, and as the algebraic sign of the difference between the two means is negative (table 3), we conclude that the value BMD (by absolute value) in the spine site was greater than in the femur neck site of the sample.

Then The Wilcoxon test was performed to study the significance of differences between the frequency of osteoporosis degree (depending on T score) in both the spine site and femur neck site of the study sample as follows:

Table 4. determination the degree of osteoporosis in the study samples according to the measurement site

BMD site	Normal BMD	Low BMD	Osteoporosis	Total
Lumber spine	20	39	21	80
Femur neck	33	42	5	80

Table 5 shows that the *P*-value less than of 0.05, meaning that at the 95% confidence level there are statistically significant differences between the recurrences of osteoporosis degree in the spine and the recurrences of osteoporosis degree in the femur neck according to the T score of the sample, and by the study of ranks with an algebraic sign, the number of negative grades (in which the osteoporosis degree in femur neck was lower than the spine site) was greater than the number of positive grades (in which the osteoporosis degree in femur neck higher than the spine site), and therefore we conclude that the degree of osteoporosis according to the T score in the spine site was higher than in the femur neck site of the sample.

Table 5. Results of the Wilcoxon test of recurrences of osteoporosis degree between spine site and femur neck site of the study sample

Comparison of osteoporotic degree depending on T score between two sites	z-value	*P*
Lumber spine-femur neck	-5.048	0.00001

Discussion

Bone is a highly dynamic tissue that is constantly changing in response to biochemical and mechanical signals throughout life, and thus the effects of risk factors on bone health could vary according to biological differences at each stage of bone metabolism (9). In adults, the entire skeleton is replaced in about every 10 years in a process called bone remodeling. Two major cell types are involved in bone remodeling: osteoclasts that resorb bone tissue and osteoblasts that actively synthesize new bone to fill the resulting lacunae (10).

Osteoporosis is the most common metabolic bone disorder and remains an increasingly significant problem, affecting 200 million individuals worldwide. Osteoporosis often is undertreated and under-recognized, in part because it is a clinically silent disease until it manifests in the form of fracture (1).

Postmenopausal osteoporosis is a systemic bone metabolism disorder affecting 30% of women over the age of 50. Among postmenopausal white women, the lifetime risk of hip fracture is 15–20% and the risk of any osteoporotic fractures is about 50% (10).

In women, osteoporosis and fractures occur mainly as a consequence of postmenopausal estrogen deficiency and an imbalance between bone resorption by osteoclasts and bone formation by osteoblasts, leading to bone loss with each remodeling cycle (11).

The menopausal transition is a complex process, in which, besides hormonal shifts, iron metabolism is also altered. Parallel with the decline in estrogen level, a two-to three-fold increase in serum ferritin concentration was detected in postmenopausal. Although elevated iron as a result of menopause is in the physiologic range, mounting evidence suggests that besides estrogen deficiency, iron/ferritin accumulation affects the health of postmenopausal women (10).

Ferritin is a ubiquitous intracellular protein that provides stored iron in a non-toxic form, and ferritin levels reflect the amount of iron stored in the body. Previously mentioned, serum ferritins increase by 2-3 times during the menopausal period. Given that overloaded serum ferritin, which is known to be a source of decrease in BMD, is more profound in postmenopausal women, we speculated that an increase in serum ferritin would cause more cases of osteoporosis in postmenopausal women (12).

In the previous decade, research into iron metabolism and bone metabolism have progressed rapidly; the results of which have improved the

understanding of the pathogenesis underlying PMOP The maintenance of iron homeostasis in postmenopausal women has been recognized as crucial and indicates the therapeutic potential of the manipulation of iron levels for treating PMOP (13).

Nonetheless, a number of studies found a negative association between SF and BMD. Chon et al. discovered a trend toward decreased LS-BMD only in premenopausal but not in postmenopausal women, whereas there was no association between SF and FNBMD. Similarly, Kim et al., in a population-based cross-sectional study found an inverse association of SF with BMD at all measurement sites only in women > 45 years of age and an increased odds of prevalent osteoporosis and fractures in high SF quartiles. A similar negative association between SF with LSBMD and FNBMD has been reported in women aged > 48 years. In a study of women with hip fractures, SF > 150 ng/ml was associated with L BMD, and SF was negatively correlated with both FN-BMD and LSBMD and positively with bone turnover markers (14).

In addition, our study shows that spine BMD is lower than the femur BMD, where the percentage of osteoporosis was 26.3% in the lumbar spine and 6.3% in the femur neck. This result coincided with the results of the Kyung- Shik Lee et al. study in Korea in 2014, which found that the prevalence of osteoporosis in the lumbar spine in women over the age of 50 years greater than in the femur neck, and the percentage was 24.4% and 19.2%, respectively (15). In Cheng XG et al. study in 2007, the incidence of osteoporosis in the lumbar spine of women over e 50 years was 28%, and in the femur neck 15%, i.e., the prevalence of the back vertebrae was greater than in the hip, and the results were consistent with the results of our study (16). These results explain that bone loss in women over 50 years is more severe in the lumbar spine. While our results not compatible with the results of the M Aghaei study in Iran 2012, which found that the number of women with osteoporosis, over 40 years of age, in the hip joint was greater than those in the lower back (17). The difference between our study and this study may be due to the fact that the prevalence of osteoporosis varies according to race, nature of life, and dietary intake.

Finally, the outcome of our study shows a decrease in BMD as serum ferritin levels increased in postmenopausal women, especially on women> 60. Serum ferritin was proved by the linear regression model to be an independent risk for reduced T score at both hip and lumbar vertebrae. This relation was not evident for BMD in the regression model. It could be

attributed to the small sample size of the studied population. More research is needed to address this relation.

Serum ferritin plays an important role in different organ functions and related pathology. Our study suggests that high serum ferritin levels are associated with low BMD in the lumbar spine and femur neck and may provide additional information for predicting poor bone health outcomes in postmenopausal women. Further interventional studies are needed to confirm the causal role of body iron stores in humans.

Acknowledgments

References

1. Lin JT, Lana JM. Osteoporosis: areview. Clin Orthop Relat Res 2004;425:126-34.

2. Juliana M. Kling, B.L.C, Nicole P. Sandhu. Osteoporosis Prevention, Screening, and Treatment: A Review J Womens Health 2014;23:563-72.

3. Proff P, Romer P. The molecular mechanism behind bone remodelling. Clin Oral Investig 2009;13:355-62

4. Nupur Kohli a, S.H.a., Stuart J. Brown a, Prasad Sawadkar a, Vaibhav Sharma a, Martyn Snowb, et al, Bone remodelling in vitro Where are we headed. Bone 2018;110:38-46.

5. Watts NB. Postmenopausal Osteoporosis a clinical review. J Womens Health 2018;27:1093-6.

6. Qian Cheng, Xiaofei Zhang, Jun Jiang, Guoyang Zhao, Yin Wang, Youjia Xu, et al. Postmenopausal Iron Overload Exacerbated Bone Loss by Promoting the Degradation of Type I Collagen. Hindawi, BioMed Research International 2017;2017:1-9.

7. Laura Toxqui, M. Pilar Vaquero .Chronic Iron Deficiency as an Emerging Risk Factor for Osteoporosis A Hypothesis. Nutrients 2015;7:2324-2344.

8. Knovich MA1, Storey JA, Coffman LG, Torti SV, Torti FM. Ferritin for the Clinician Blood Rev. 2009;23:95-104.

9. Ahn SH, L.S., Kim H, Lee SH, Kim BJ, Koh JM. Higher serum ferritin level and lower femur neck strength in women at the stage of bone loss ($\geq$ 45 years of age): The Fourth Korea National Health and Nutrition Examination Survey (KNHANES IV). Endocr Res 2016;41:334-42.

10. Jeney V.Clinical Impact and Cellular Mechanisms of Iron Overload-Associated Bone Loss. Front Pharmacol 2017;8:77.

11. Brandão CM1, Lima MG, Silva AL, Silva GD, Guerra AA Jr, Acúrcio Fde A. Treatment of postmenopausal osteoporosis in women: a systematic review. Cad Saude Publica 2008;24:592-606

12. Chon SJ, Choi YR, Roh YH, Yun BH, Cho S, Choi YS, et al. Association between Levels of Serum Ferritin and Bone Mineral Density in Korean Premenopausal and Postmenopausal. PLoS One. 2014;9:114972.

13. Chen B, Li GF, Shen Y, Huang XI, Xu YJ. Reducing iron accumulation A potential approach for the prevention and treatment of postmenopausal osteoporosis. Exp Ther Med 2015;10:7–1

14. Mansour Babaei, Ali Bijani, Parnaz Heidari, Seyyed Reza Hosseini, Behzad Heidari. Serum ferritin levels and bone mineral density. Caspian J Intern Med 2018;9: 232-8.

15. Lee KS, Bae SH, Lee SH, Lee J, Lee DR. New Reference Data on Bone Mineral Density and the Prevalence of Osteoporosis in Korean Adults Aged 50 Years or OlderThe Korea National Health and Nutrition Examination Survey 2008 2010. J Korean Med Sci 2014;29:1514-22.

16. Cheng XG1, Yang DZ, Zhou Q, Zhuo TJ, Zhang HC, Xiang J, Wang HF, et al. Age-related bone mineral density, bone loss rate, prevalence of osteoporosis, and reference database of women at multiple centers in China. J Clin Densitom 2007;10:276-84.

17. Mehrdad Aghaei, Hamid Reza Bazr Afshan, Mostafa Qorbani, Hossien Shadpour Dashti, Roya Safari. Bone mineral density in Iranian patients Effects of age sex and body mass index. Open J Prev Med 2013;3:128-31.