Do Vitamin D Levels Affect Bone Mineral Density in Reproductive Women with Low Ferritin Levels?

Düşük Ferritin Düzeyine Sahip Reprodüktif Kadınlarda D Vitamini Düzeyleri Kemik Mineral Dansitesini Etkiler mi?

Ⓓ *Hatice Hamarat,* Ⓓ *Göknur Yorulmaz*,* Ⓓ *Ayşe Ekim Aydemir**,* Ⓓ *Emel Örge Gönüllü***,* Ⓓ *Ahmet Musmul****,*
Ⓓ *Sinem Gürcü******

Eskişehir City Hospital, Clinic of Internal Medicine, Eskişehir, Turkey
*Eskişehir Osmangazi University Faculty of Medicine, Department of Endocrinology and Metabolism, Eskişehir, Turkey
**Eskişehir City Hospital, Clinic of Physical Therapy and Rehabilitation, Eskişehir, Turkey
***Sakarya University Faculty of Medicine, Department of Rheumatology, Sakarya, Turkey
****Eskişehir Osmangazi University Faculty of Medicine, Department of Statistics, Eskişehir, Turkey
*****Eskişehir City Hospital, Clinic of Pharmacy, Eskişehir, Turkey

Abstract

Objective: Iron deficiency anemia (IDA) is common in reproductive women. 25-hydroxyvitamin D [25-(OH)D] deficiency is common in those with IDA. The study evaluated the effect of low ferritin on 25-(OH)D levels and bone mineral density (BMD).

Materials and Methods: One hundred forty women (15-25 years, 25-35 years, 35-45 years, 3 groups) with ferritin level <15 ng/mL and a control group of 50 healthy women were included in the study. A vitamin D level of 25-(OH)D>30.0 ng/mL was sufficient, vitamin D deficiency was defined as 25-(OH)D≥20.0, <30.0 ng/mL. Vitamin D deficiency as 25-(OH)D<20 ng/mL. Severe vitamin D deficiency 25-(OH)D<10 ng/mL. Femoral neck and lumbar spine BMD were measured by dual-energy X-ray absorptiometry. For the diagnosis of BMD, a Z-score of <-2 was evaluated with "lower bone mineral density than expected" and "expected bone density for >-2 years of age."

Results: The mean ages of the patients and healthy controls were 34±10 and 29±9 years. Serum 25-(OH)D level was found to be low in the patient group (p<0.01). Low-intensity BMD was present in the low ferritin groups (p>0.05).

Conclusion: Low vitamin D levels detected in reproductive women with anemia cause low BMD.

Keywords: Anemia, bone formation, nutrition, metabolism, vitamins

Öz

Amaç: Reprodüktif kadınlarda demir eksikliği anemisi (DEA) sık gözlenir. 25-hidroksivitamin D [25-(OH)D] eksikliği DEA olanlarda yaygındır. Ferritin düşüklüğüne bağlı oluşan 25-(OH)D eksikliğinin kemik mineral yoğunluğu (KMY) üzerindeki etkisi tartışmalıdır. Bu çalışma, reprodüktif kadınlarda serum ferritin düşüklüğünün 25-(OH)D seviyelerine etkisi ve KMY arasındaki ilişkiyi değerlendirmek için yapıldı.

Gereç ve Yöntem: Çalışmaya ferritin seviyeleri <15 ng/mL olan 140 üreme dönemindeki kadın (15-25 yaş, 25-35 yaş, 35-45 yaş olmak üzere 3 grup) ve 50 sağlıklı kadından oluşan bir kontrol grubu dahil edildi. Vitamin D düzeyi 25-(OH)D>30,0 ng/mL ise yeterli, 25-(OH)D≥20,0, <30,0 ng/mL ise D vitamini yetersizliği, 25-(OH)D <20 ng/mL ise D vitamini eksikliği ve <10 ng/mL ise şiddetli D vitamini eksikliği olarak tanımlandı. Femur boynu ve lomber omurga KMY'si çift enerjili X-ışını absorpsiyometrisi ile ölçüldü. KMY tanısı için Z-skoru kullanıldı. Z-skoru <-2 ise "beklenenden daha düşük KMY'ye sahip" ve >-2 ise "yaş için beklenen kemik yoğunluğu" olarak değerlendirildi.

Bulgular: Hastaların ve sağlıklı kontrollerin ortalama yaşı 34±10 ve 29±9 yıldı. Serum 25-(OH)D düzeyi hasta grubunda anlamlı farklılık oluşturacak kadar düşüktü (p<0,01). Düşük ferritin gruplarında, düşük yoğunluklu KMY vardı (p>0,05).

Sonuç: Anemisi olan reprodüktif kadınlarda saptanan düşük D vitamini seviyeleri düşük KMY'ye neden olur.

Anahtar kelimeler: Anemi, kemik oluşumu, beslenme, metabolizma, vitamin

Turk J Osteoporos
2022;28:26-31

Hamarat et al.
Anemia and Bone Formation **27**

Introduction

Iron deficiency anemia (IDA) is a major health problem worldwide and accounts for half of all anemia cases. IDA is more common in young women and children of reproductive age. It causes many health problems such as maternal and infant mortality and poor physical performance (1,2). One of these problems is bone development. For bone health, some vitamins and minerals in the body must be at sufficient levels, the most important of which are vitamin D and iron levels. Iron is an essential component for all living cells. It takes part in great important metabolic processes which are vital to the cell (eg oxygen transport, DNA synthesis) (3). The amount of iron storage in the body is best shown by hemosiderin in the insoluble form found in the bone marrow (4). The most accurate method for iron fixation is to stain the bone marrow sample with Prussian blue. This is an invasive and impractical method (5). For this reason, ferritin measurement is preferred to evaluate serum iron in daily use (6,7). Iron is an essential factor for enzymes associated with bone structure. Hydrolases in the collagen structure contain iron (8). Also, iron is used as a cofactor in transforming vitamin D into its active form (9). In rats with iron deficiency, it was shown that bone mineral content, bone mineral density (BMD) which is associated with bone strength, were reduced (10,11). Therefore, it was thought that the decrease in iron affects bone structure. Katsumata et al. (12) in his study, the rate of bone formation decreased in rats due to serum vitamin D [25-hydroxyvitamin D (25-(OH)D)]. Many researchers studied the relation between iron in the dietary content and bone structure. In human studies, it was determined to ferritin has a positive contribution to BMD in postmenopausal women (13,14). Red meat is a source of both iron and vitamin D. Ferritin was found to be quite low in vegetarians (15). Similarly, in an another study, plasma 25-(OH)D was found to be high in those who consume meat and sea products a lot, while it was found to be low in vegetarians and vegans (16). In a systematic review, it is shown that bone density and fracture risk are investigated in those fed fish, seafood, other meat products. And it has been reported that protein obtained from fish or other meat species contributes significantly to bone structure (17). Disorders in the bone structure and development process cause serious irreversible loss of work power, impairment of quality of life, disabilities at older ages. IDA is a preventable and treatable health problem. In the bone development process, its negative effects on the bone and other organs can be prevented with adequate and effective treatment. In this study, it is aimed to elucidate the effect of low ferritin levels on vitamin D metabolism and bone formation.

Materials and Methods

Participants

In this study, 140 female patients (aged 15-45) in reproductive period diagnosed with IDA and 50 healthy female (aged 15-45) subjects as a control group were assessed in the internal medicine department. IDA patients were selected from those who were diagnosed at least one year ago but did not receive regular treatment. Infectious, inflammatory diseases, malignancy patients, liver diseases, chronic kidney patients and blood transfusion, patients who took parenteral or oral iron supplements a month ago were not included in the study. Also, those with a chronic disease, drug use, previously diagnosed with osteopenia or osteoporosis, receiving a vitamin D supplement, whose 25-(OH)D levels were 30 ng/mL and higher were excluded. All participants were selected from women with regular menstruation. Patients in the study were divided into 3 groups: group 1 (15-25 age years), group 2 (26-35 age years), group 3 (36-45 age years) to evaluate vitamin D and BMD more accurately. A written acceptance certificate was requested from the patients included in the study. The research protocol was authorized by the Institutional Ethics Committee. Research permission was obtained from Eskişehir Osmangazi University Non-Interventional Clinical Research Ethics Committee (decision no: 30, date: 18.02.2020).

Laboratory Evaluation

In whole blood, blood samples containing hematocrit (HCT), hemoglobin (Hb) concentration (Hb), ferritin, 25-(OH)D levels tested in the laboratory (fasting for at least six hours and in the morning between 08:00-09:00). Samples were collected in EDTA tubes for whole blood count and tested with the automatic hematology analyzer. Ferritin concentration was measured with the latex-enhanced immunoturbidimetric method. Serum vitamin D levels were measured using a Chromosystem assay [Abbott Architect i2000SR analyzer and Architect 25-OH Vitamin D kit (Abbott laboratories, USA, Spain)] using the high performance liquid chromatography method. Vitamin D levels were measured in the November-January period. Vitamin D level was defined as 25-(OH)D>30.0 ng/mL adequate, vitamin D insufficiency as 25-(OH)D ≥20.0, <30.0 ng/mL, vitamin D deficiency as 25-(OH)D<20 ng/ mL. and <10 ng/mL. Severe vitamin D deficiency (3) Hb, HCT, ferritin were studied for iron deficiency. The World Health Organization (WHO), has reported a Hb level of <12 ng/dL in woman with anemia (18). IDA is also determined with a mean corpuscular volume of <80 and a serum ferritin level of <15-30 µg/L. A ferritin threshold value for the diagnosis of iron deficiency was accepted as 15 µg/L (19,20). Dual-energy X-ray absorptiometry (DXA) device was used to evaluate the BMD of the lumbar spine and femoral neck. The BMD of the patients was measured on anteroposterior and lateral lumbar vertebrae (L1-L4) and left femur neck. Measurements were made with DXA (FDX Visionary-DR, H105 138-SN:E18 7H 0487) in the radiology department. Filming was completed in 5-15 minutes and BMD results were expressed as g/cm^2, as Z-score. Z-score is the standard deviation (SD) of BMD for the individual's age group. BMD results were evaluated according to the Z-score according to the criteria recommended by the WHO. Those with a Z-score <-2 were evaluated as "having low BMD" and those

28 Hamarat et al.
Anemia and Bone Formation

Turk J Osteoporos
2022;28:26-31

with a Z-score of> -2 as "having expected bone density for age" (21).

Statistical Analysis

Research data analyzed with SPSS version 22.0. Nominal variables were expressed as mean ± SD. The data consisting of independent measurements that did not show normal distribution were evaluated with the Mann-Whitney U test. Datasets in the categorical structure were evaluated with chi-square tests. A value of p<0.05 was considered statistically significant.

Results

One hundred fourty women with low ferritin levels and 50 healthy women as a control group participated in the study. Demographic and laboratory data of patients and control groups are presented in Table 1. In the laboratory evaluation of patients, calcium, phosphorus, parathyroid hormone, alanine aminotransferase, alkaline phosphatase, creatinine, follicle-stimulating hormone values were within normal limits. Serious vitamin D deficiency was detected in all age groups of the low ferritin group and there was a significant statistical difference was found between the control group (p<0.01). Vitamin D deficiency was more common in group 2, group 3 and in the control group (p<0.01). Femoral neck Z-score measurement was normal in all groups included in the study. Therefore, lumbar vertebra Z-score measurements were used in the study. BMD value was normal in 50.7% of the low ferritin group, 49.2% had lower BMD than expected for age. BMD was lower than expected in 38% of the

control group. BMD was lower in all age groups in the patient group and did not make a statistical difference between this control group (p>0.05). In group 2 (26-35 age), the frequency of low BMD was higher in the patient group and made a statistical difference (p<0.01). In the control group, only in group 2, there was a decrease in BMD (p<0.01). Vitamin D levels and BMD ratios for all age groups and the relationship between them are shown in Table 2 and 3.

Discussion

We investigated serum ferritin and vitamin D levels and BMD measurements in women of reproductive age with low ferritin and normal ferritin. After adjusting for variables, some important findings were obtained. Low levels of 25-(OH)D were detected at low ferritin levels. The relationship between ferritin and 25-(OH)D has been the subject of research in many studies. Jeong et al. (22) and Andıran et al. (23) found 25-(OH)D levels were associated with ferritin levels in their study in the USA and Korea. Studies conducted in the USA and Portugal have reported that there is no relationship between serum ferritin and 25-(OH)D levels in adults (24,25). These different findings in studies may be depending on many factors such as studies in different races and ethnic societies, personal nutrition habits, geography, and work environment. Lee et al. (26) found serum ferritin value as <12 in healthy Korean women with vitamin D <15 ng/mL. Suh et al. (27) found in their study in the Korea that the level of vitamin D was lower in women with low serum ferritin (<15 μg/L) than those with a normal serum ferritin level (≥15 μg/L) (p<0.001). In this study, we found vitamin D deficiency in all groups. Serious

Table 1. Demographic and laboratory data of patients							
Variable*	**Patients Mean ± SD**			**Control Mean ± SD**			**p-value**
	G1	**G2**	**G3**	**G1**	**G2**	**G3**	
	(15-25) n=32	**(26-35) n=50**	**(36-45) n=58**	**(15-25) n=14**	**(26-35) n=24**	**(36-45) n=12**	
Age (years)	33.56±9.7			28.92±8.8			=0.088
All years	24±9.8	34±9.9	43±8.8	20±8.5	30±8.1	37±8.1	
Hb (g/dL)	9.84±1.9			12.38±1.1			<0.001
HCT (%)	31.66±4.6			36.20±3.9			<0.001
Ferritin (μg/L)	3.92±3.1			34.72±15.9			<0.001
25-(OH)D (ng/mL)							
<10.0	7.42±6.4			10.35±3.3			<0.001
≥10.0, <20.0	11.14±1.2			15.32±4.1			<0.001
≥20.0, <30.0	22.12±2.4			23.52±2.5			>0.05
BMD lumbal Z-score							
<-2	-2.42±2.1			-2.12±1.1			>0.05
>-2	1.72±1.4			2.01±1.2			>0.05
*Variable: Group 1-2-3 abbreviated as G1, G2, G3, SD: Standard deviation, Hb: Hemoglobin, HCT: Hematocrit, BMD: Bone mineral density, 25-(OH)D: 25-hydroxyvitamin D, Mann-Whitney U test and chi-square tests applied.							

Turk J Osteoporos
2022;28:26-31

Hamarat et al.
Anemia and Bone Formation **29**

Table 2. Classification by age groups BMD and D vitamin levels									
Variable*	**Patients**			**Control**			**p-value**		
	G1 (15-25) (%)	**G2 (26-35) (%)**	**G3 (36-45) (%)**	**G1 (15-25) (%)**	**G2 (26-35) (%)**	**G3 (36-45) (%)**	**G1**	**G2**	**G3**
25-(OH)D (ng/mL)									
<10.0**	81.0	84.0	75.0	57.0	54.0	50.0	<0.01	<0.01	<0.01
≥10.0, <20.0**	19.0	16.0	23.0	43.0	46.0	48.0	<0.01	<0.01	<0.01
≥20.0, <30.0	0.0	02.0	0.0	0.0	0.0	02.0	>0.05		
BMD lumbal Z-score									
<-2**	53.0	52.0	45.0	42.0	33.0	42.0	>0.05	<0.01	>0.05
>-2**	47.0	48.0	55.0	58.0	68.0	58.0	>0.05	<0.01	>0.05
*Variable: Group 1-2-3 abbreviated as G1, G2, G3, **The p-value shows the statistical significance between the same age group, Mann-Whitney U test and chi-square tests applied, BMD: Bone mineral density, 25-(OH)D: 25-hydroxyvitamin D									

Table 3. Comparison between vitamin D levels and bone mineral densitometry						
Variable	**BMD lumbal Z-score**				**p-value**	
	Patients Mean ± SD		**Control Mean ± SD**			
	<-2	**>-2**	**<-2**	**>-2**	**<-2**	**>-2**
25-(OH)D (ng/mL)						
<10.0	-2.51±1.1	-1.45±2.3	-2.23±2.1	1.83±1.4	>0.05	<0.01
p-value	<0.001		<0.001			
≥10.0, <20.0	-2.33±1.1	2.12±2.4	-2.01±1.4	2.07±1.6	>0.05	>0.05
p-value	<0.001		<0.001			
≥20.0, <30.0	-	2.59±1.3	-	2.24±1.3	>0.05	
SD: Standard deviation, BMD: Bone mineral density, 25-(OH)D: 25-hydroxyvitamin D, Mann-Whitney U test and chi-square tests applied						

vitamin D deficiency and low-density BMD values were found in the low ferritin group. Studies are showing that low iron levels can cause low BMD. Hematopoietic growth factors are secreted by with blood loss, and osteoclasts increase. Thus, progenitor cell proliferation begins in the bone marrow. Osteoclasts initiate bone destruction. One of the cells that increase due to blood loss is osteoblasts. Osteoblasts increase bone formation while osteoclasts increase destruction. Therefore, conditions that cause recurrent blood loss, such as female menstrual bleeding, can reduce BMD by increasing osteoblast and osteoclast production (26). In this study, low-intensity BMD measurement was more common in all age groups of the low ferritin group, but this did not make a significant difference with the control group (p>0.05). Low BMD frequency in group 2 (26-35 age), patient group, and normal BMD frequency in the control group were statistically different (p<0.01). Based on all these, the low level of BMD is associated with peak bone mass formation. 90% of the peak bone mass is reached until the age of 18. They are all reached around the age of 30. In most women, bone mass remains constant until menopause. BMD starts to decrease with loss of estrogen with menopause and aging (28). BMD normality was highest in the low ferritin group between the ages of 36-

45. This showed an improvement in BMD measurement due to an increase in peak bone mass as age increased, regardless of vitamin D deficiency. Despite the small group of people in this study, it can be said that low ferritin levels can cause low vitamin D levels and this negatively affects bone density. However, we believe that variables such as genetic factors, body mass index (BMI), and diet should be taken into account, these may be the shortcomings of our study. In both groups, the lack of D vitamin level can be attributed to reasons such as poor diet in the process from the beginning of the reproductive period to the end, lack of direct contact with the sun, and increased alcohol and cigarette use (29,30).

The limitations of these studies are the inability to inquire about the factors affecting vitamin D and ferritin levels, such as nutritional diversity, smoking and alcohol use, weight and height measurement, BMI, working in a closed environment. The relationship between ferritin, vitamin D and bone mass will become clear with multicenter studies with more patient participation. We believe that our study will raise awareness about the decrease in bone mass in early ages and will lead to more extensive research on this subject.

30 Hamarat et al.
Anemia and Bone Formation

Turk J Osteoporos
2022;28:26-31

Conclusion

Low ferritin levels in women of reproductive age can lead to low BMD measurements and may be considered to affect bone adversely with different mechanisms. Recurrent iron loss increases bone loss in patients and may cause osteoporosis and fracture risk, but more comprehensive studies are needed. This situation should also be evaluated in terms of genetics. If reproductive patients with recurrent IDA experience muscle and joint pain, BMD measurement should be performed. Patients with low BMD should be followed to prevent known complications. Women aged 18-50 may be advised to take calcium and vitamin D daily. Other lifestyle suggestions, regular load-bearing exercises (such as walking), avoiding smoking alcohol consumption, and limiting caffeine consumption should be recommended.

Ethics

Ethics Committee Approval: Research permission was obtained from Eskişehir Osmangazi University Non-Interventional Clinical Research Ethics Committee (decision no: 30, date: 18.02.2020).
Informed Consent: A written acceptance certificate was requested from the patients included in the study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.H., A.E.A., E.Ö.G., Concept: H.H., G.Y., E.Ö.G., Design: H.H., G.Y., A.E.A., Data Collection or Processing: H.H., A.E.A., A.M., S.G., Analysis or Interpretation: H.H., A.M., Literature Search: H.H., G.Y., E.Ö.G., Writing: H.H., G.Y., S.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. Lancet 2016;387:907-16.

2. Cohen A, Shane E. Treatment of premenopausal women with low bone mineral density. Curr Osteoporos Rep 2008;6:39-46.

3. Conrad ME, Umbreit JN, Moore EG. Iron absorption and transport. Am J Med Sci 1999; 318:213-29.

4. Anand T, Rahi M, Sharma P, Ingle GK. Issues in prevention of iron deficiency anemia in India. Nutrition 2014;30:764-70.

5. Phiri KS, Calis JCJ, Kachala D, Borgstein E, Waluze J, Bates E, et al. Improved method for assessing iron stores in the bone marrow. J Clin Pathol 2009;62:685-9.

6. Fernandez-Rodriguez AM, Guindeo-Casasus MC, Molero-Labarta T, Dominguez-Cabreraet C, Hortal-Casc n L, Perez-Borges P, et al. Diagnosis of iron deficiency in chronic renal failure. Am J Kidney Dis 1999;34:508-13.

7. KDOQI;National Kidney Foundation. KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease. Am J Kidney Dis 2006;47(Suppl 3):11-145.

8. Tuderman L, Myllyla R, Kivirikko KI. Mechanism of the prolyl hydroxylase reaction. 1. Role of co-substrates. Eur J Biochem 1977;80:341-8.

9. DeLuca HF. Metabolism of vitamin D: current status. Am J ClinNutr 1976;29:1258-70.

10. Medeiros DM, Plattner A, Jennings D, Stoecker B. Bone morphology, strength and density are compromised in iron-deficient rats and exacerbated by calcium restriction. J Nutr 2002;132:3135-41.

11. Medeiros DM, Stoecker B, Plattner A, Jennings D, Haub M. Iron deficiency negatively affects vertebrae and femurs of rats independently of energy intake and body weight. J Nutr 2004;134:3061-7.

12. Katsumata S, Katsumata-Tsuboi R, Uehara M, Suzuki K. Severe iron deficiency decreases both bone formation and bone resorption in rats. J Nutr 2009;139:238-43.

13. Harris MM, Houtkooper LB, Stanford VA, Parkhill C, Weber JL, Flint-Wagner H, et al. Dietary iron is associated with bone mineral density in healthy postmenopausal women. J Nutr 2003;133:3598-602.

14. Maurer J, Harris MM, Stanford VA, Lohman TG, Cussler E, Going SB, et al. Dietary iron positively influences bone mineral density in postmenopausal women on hormone replacement therapy. J Nutr 2005;135:863-9.

15. Śliwińska A, Luty J, Aleksandrowicz-Wrona E, Małgorzewicz S. Iron status and dietary iron intake in vegetarians. Adv Clin Exp Med 2018;27:1383-9.

16. Crowe FL, Steur M, Allen NE, Appleby PN, Travis RC, Key TJ. Plasma concentrations of 25-hydroxyvitamin D in meat eaters, fish eaters, vegetarians and vegans: results from the EPIC-Oxford study. Public Health Nutr 2011;14:340-6.

17. Perna S, Avanzato I, Nichetti M, D'Antona G, Negro M, Rondanelli M. Association between Dietary Patterns of Meat and Fish Consumption with Bone Mineral Density or Fracture Risk: A Systematic Literature. Nutrients 2017;9:1029.

18. World Health Organization. Nutritional Anaemias. Report of a WHO Scientific Group. World Health Organ Tech Rep Ser Geneva, Switzerland; 1968;405:5-37.

19. Camaschella C. Iron-deficiency anemia. N Engl J Med 2015;372:1832-43.

20. Pasricha SR, Flecknoe-Brown SC, Allen KJ, Gibson PR, McMahon LP, Olynyk JK, et al. Diagnosis and management of iron deficiency anaemia: A clinical update. Med J Aust 2010;193:525-32.

21. Khosla S, Lufkin EG, Hodgson SF, Fitzpatrick LA, Melton LJ 3rd. Epidemiology and clinical features of osteoporosis in young individuals. Bone 1994;15:551-5.

22. Jeong DW, Lee HW, Cho YH, Yi DW, Lee SY, Son SM, et al. Comparison of serum ferritin and vitamin D in association with the severity of nonalcoholic fatty liver disease in Korean adults. Endocrinol Metab (Seoul) 2014;29:479-88.

23. Andıran N, Çelik N, Akça H, Doğan G. Vitamin D deficiency in children and adolescents. J Clin Res Pediatr Endocrinol 2012;4:25-9.

24. Monlezun DJ, Camargo CA Jr, Mullen JT, Quraishi SA. Vitamin D status and the risk of anemia in community-dwelling adults: results from the National Health and Nutrition Examination Survey 2001-2006. Medicine (Baltimore) 2015;94:e1799.

25. Castro FD, Magalhães J, Carvalho PB, Moreira MJ, Mota P, Cotter J. Lower levels of vitamin D correlate with clinical disease activity and quality of life in inflammatory bowel disease. Arq Gastroenterol 2015;52:260-5.

26. Lee JA, Hwang JS, Hwang IT, Kim DH, Seo JH, Lim JS. Low vitamin D levels are associated with both iron deficiency and anemia in children and adolescents. Pediatr Hematol Oncol 2015;32:99-108.

27. Suh YJ, Lee JE, Lee DH, Yi HG, Lee MH, Kim CS, et al. Prevalence and relationships of iron deficiency anemia with blood cadmium and vitamin D levels in Korean women. J Korean Med Sci 2016;31:25-32.

28. Riggs BL, Melton LJ 3rd. Involutional osteoporosis. N Engl J Med 1986;314:1676-86.

29. Ross AC, Taylor CL, Yaktine AL,Valle HB. Dietary Reference Intakes for calcium and vitamin D. Washington(DC):National Academies Press(US); 2011. https://doi.org/10.17226/13050

30. McLendon AN, Woodis CB. A Review of Osteoporosis Management in Younger Premenopausal Women. Women's Health (Lond) 2014;10:59-77.