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Research Article

Association between Serum Vitamin B12 Level and Bone Mineral Density in Older Women

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Abstract

Background: Vitamin B12 is a micronutrient essential for deoxyribonucleic acid (DNA) synthesis, which can affect osteogenesis. Based on in-vivo investigations, vitamin B12 is associated with osteogenesis, and low levels of this vital vitamin in the human body can be related to an increased risk of osteoporosis.
Methods: In this descriptive-analytical study, 60 women over 65 years who visited an orthopedic clinic were included. They were divided into three groups based on bone density in the hip and lumbar areas using dual-energy X-ray absorptiometry (DEXA) scans, including normal bone density, osteopenia, and osteoporosis. The average serum level of vitamin B12 in the experimental subjects was then determined and compared.
Results: 6 (10%) showed normal bone density, 25 (41.7%) osteopenia, and 29 (48.3%) were considered to have osteoporosis. Measured vitamin B12 levels showed no statistically significant difference between the two groups of normal bone density (601.3 ± 194.8) and osteopenia (560.4 ± 131.5). However, there was a significant statistical difference between vitamin B12 levels in people with osteoporosis (400.7 ± 162.4) and the two groups of normal and osteopenic individuals. There was also a negative statistical relationship between vitamin B12 levels and bone density (P = 0.004, r = -0.8).
Conclusion: A low serum level of vitamin B12 is associated with a severe decline in bone density in elderly Iranian women.

Keywords: Bone Density; Bone Diseases; Osteoporosis; Vitamin B12

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Background

Vitamin B12 is an indispensable micronutrient for deoxyribonucleic acid (DNA) synthesis and can affect osteogenesis. In clinical and cellular research, vitamin B12 is considered to be related to osteogenesis, and its low level in the body can be related to a high risk of osteoporosis (1). There are several reasons why older people are a high-risk population for nutrient deficiencies. Some of these causes include age-related physiological changes, chronic diseases, and medication overuse. Since cobalamin absorption is often declined in the elderly, vitamin B12 deficiency is one of the most important nutritional problems in this population (2-5). Recent studies suggest that inadequate vitamin B12 levels can promote the risk of atherosclerotic and neurodegenerative diseases (3, 6-9).

Osteoporosis is the most common metabolic bone disease, which is related to decreased bone mass and increased bone brittleness (10, 11). Although osteoporosis causes morbidity and mortality, it can be prevented. In recent years, much research has been conducted to investigate the association between hyperhomocysteinemia, as well as folate and vitamin B12 deficiencies, and decreased bone mass (12). Two fundamental substances in the methionine metabolism pathway are B12 and folate (12, 13). Studies have shown a positive association between serum B12 and folate levels with bone mineral densitometry (BMD) and a negative association between hyperhomocysteinemia and BMD (14-16). Concomitant consumption of folate and vitamin B12 supplements reduces the risk of hip fractures in the elderly with hemiplegia following a stroke. Lower concentrations of vitamin B6 are related to further bone loss (17, 18). Low plasma concentrations of vitamin B12 explain the association between low plasma homocysteine (Hcy) and increased risk of hip fracture (19). Studies have indicated that a normal level of B12 is protective of maintaining femoral neck bone density in older men. Osteoporosis is a major public health problem in the elderly, and most types of osteoporotic fractures increase with aging (20). The association between increased vitamin B12 levels in people with osteoporosis compared to those with normal BMD may be due to genetic differences (13).

Few studies have been done on the relationship between vitamin B12 and osteoporosis worldwide, but there have not been any studies in Iran on this issue, and this is the first study to investigate this association in Iran. This study aimed to investigate the relationship between bone density and vitamin B12 levels in older women over 65 years.

Methods

This study was approved by the Ethics Committee of Urmia University of Medical Sciences, Urmia, Iran (IR.UMSU.REC.1397.386). In this descriptive-analytical study, 60 women over 65 who visited an orthopedic clinic were enrolled. The power of the study was 80%. The study's entry criteria were people's consent to participate in this study. After explaining the conditions of the study, the individuals entered the study if they were willing to

participate. Exclusion criteria included taking vitamin B12-containing supplements in the past three months. Patients did not receive any nutritional supplements until the fracture. According to the history taken, they had the same nutritional level and were located in the same geographical area (West Azerbaijan, Iran).

These individuals were examined for bone density in the hip and lumbar region using the conventional dual-energy X-ray absorptiometry (DEXA) scanning method, based on which the T-score was estimated. In this test, scores up to -1.5 were considered normal, -1.5 to -2.5 were considered osteopenia, and -2.5 and lower were considered osteoporosis. Then, serum vitamin B12 levels were measured in a standard laboratory for all these patients (by electrochemiluminescence immunoassay (ECLIA) method examined with Roche kit).

Patients were divided into three groups with normal bone density, osteopenia, and osteoporosis, and average serum levels of vitamin B12 in them were determined and compared. The normal range of serum levels of vitamin B12 is 200 to 950 picograms per milliliter (pg/ml) (14). Their heights were measured by a wall tape with an accuracy of 0.1 cm and weights by a Seca weighing machine (Seca Medical, Massachusetts, USA) with an accuracy of 0.5 kg. Then, their body mass index (BMI) was calculated in kg/m^2.

This study used descriptive statistical methods for statistical analysis, including mean, standard deviation (SD), frequency, and percentage. To compare the serum levels between the three groups, analysis of variance (ANOVA) statistical test and appropriate post hoc test (Tukey's b) were used. The Pearson correlation test was used to investigate the relationship between mean B12 level and BMD. A P-value of less than 0.05 was considered significant in this study, and SPSS software (version 20, IBM Corporation, Armonk, NY, USA) was used to analyze the data.

Results

In this study, 60 female patients over 65 years with an average age of 74 were divided into three groups based on bone density. 6 cases (10%) had a normal bone density, 25 cases (41.7%) were considered to have osteopenia, and 29 cases (48.3%) had osteoporosis. Vitamin B12 measurements showed no statistical differences between the two groups of people, the group with normal bone density and the group with osteopenia. However, post hoc analysis showed a statistically significant difference between vitamin B12 levels in people with osteoporosis and the two groups of normal and osteopenic individuals (Table 1).

Table 1. Comparing laboratory findings in three groups of patients (divided based on bone density)

Variable	Normal BMD (n = 6)	Osteopenia (n = 25)	Osteoporosis (n = 29)	P-value
Age (year)	73.10 ± 4.30	74.40 ± 5.06	74.80 ± 6.50	0.80
BMI (kg/m^2)	23.20 ± 2.10	22.40 ± 1.80	22.50 ± 2.40	0.40
BMD (T score)	-1.20 ± 0.20	-2.10 ± 3.30	-3.06 ± 0.20	0.04*
Vitamin B12 (pg/ml)	601.30 ± 194.80	560.40 ± 131.50	400.70 ± 162.40	0.03*

Data are presented as mean ± standard deviation (SD)
*Significant difference
BMI: Body mass index; BMD: Bone mineral densitometry

As shown in figure 1, the mean level of vitamin B12 measured in people with osteoporosis was lower than in the two groups of normal and osteopenic people. There was also a negative statistical relationship between vitamin B12 levels and bone density (P = 0.004, r = -0.8).

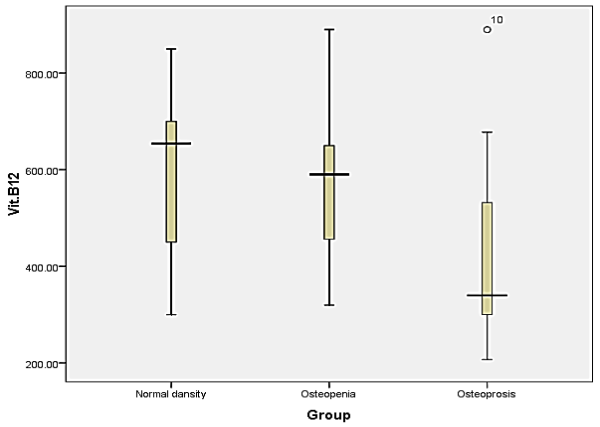

Figure 1. Comparing the average vitamin B12 in three groups of patients (based on bone density)

Discussion

This study investigated the relationship between serum levels of vitamin B12 in the blood and BMD in elderly women who visited a clinic. Our findings indicated that vitamin B12 deficiency affected patients with severe bone loss, and it was reported in people with osteoporosis more than others.

Vitamin B12 deficiency is a major problem in older people's nutrition. Recent studies suggest that insufficient vitamin B12 can be associated with an increased risk of atherosclerotic and neurodegenerative diseases (2-5). Numerous nutritionists recommend monitoring B12 status in the elderly to identify people at risk in the early stages (21).

Lower concentrations of vitamin B6 are related to further bone loss. Low concentrations of vitamin B12 in plasma justify the association between low plasma Hcy and a high risk of hip fracture (19). Studies have shown that standard B12 level plays a protective role in sustaining femoral neck bone density in older men. Osteoporosis is a considerable public health problem in the elderly population, and a vast majority of different osteoporotic fractures rise with aging (20). According to statistics, 30% of women may have osteoporosis in some stages of their lives, and osteoporosis is an economic burden on both society and families (22, 23).

Our results showed that the mean serum levels of vitamin B12 in women with osteopenia and the group with normal bone density were approximately the same. However, it was lower in women with osteoporosis and was significantly different from the other two groups. BMD levels were significantly different in these three groups. There was a significant association between vitamin B12 serum levels and BMD in women aged 65 or more, and Pearson's correlation coefficient showed a negative relationship between serum levels of vitamin B12 and BMD. In a meta-analysis by Zhang et al. about the association between Hcy, vitamin B12, and folate with bone mineral density in postmenopausal women, it was shown that folate levels in postmenopausal women with osteoporosis group were lower than in the control group. However, there was no significant difference between them. The association of Hcy and vitamin B12 with BMD was positive in postmenopausal women. The results of this meta-analysis suggest that Hcy and vitamin B12 are associated with a significantly increased risk of

osteoporosis in the future (24).

Consumption of vitamin B12 as a dietary supplement has been shown to be protective in osteoporotic postmenopausal women. However, some studies have shown that vitamin B12 has no significant relationship with BMD (13, 29). In another study, folate and vitamin B12 serum levels were significantly associated with BMD. Moreover, a possible link between folate and bone health has been reported in some studies (13). Folate is involved in bone maintenance and repair by providing a methyl group for DNA synthesis (25).

Bozkurt et al. have reported that vitamin B12 is associated with osteoporosis, similar to our results (26). In addition, You et al. showed that low levels of vitamin B12 were positively associated with decreased bone density in the hip area and its deficiency was probably a serious risk factor to develop osteoporosis in postmenopausal women (27). In addition, in a study by Zhang et al., this vitamin levels are inversely related to BMD. It seems that the association between increased vitamin B12 levels in people with osteoporosis compared to normal BMD individuals in this study can be because of genetic differences in subjects (24).

In a study by Selhub, it is shown that the total concentration of Hcy is negatively related to absorption and folate and vitamin B12 levels in plasma (28). It appears that in the absence of vitamin B12 and folate, methyl-tetrahydrofolate (MTHF) and DNA synthesis will be impaired; as a result, vitamin B12 deficiency will lead to high concentrations of Hcy. Herrmann et al. reported that an increase in concentration of Hcy stimulated bone activity, resulting in increased bone resorption (29).

In our study, only the level of vitamin B12 in people who visited an orthopedic clinic was checked. Most of them had an underlying disease, which may impact their vitamin B12 levels. However, by determining the specific entry criteria for individuals to enter the study, we have tried to match the study subjects. Moreover, vitamin D levels have not been measured and compared; as we know, concomitant vitamin D and B12 deficiency can be an effective factor too. Another limitation of our study was including just a small community of older women, which may affect our results.

Conclusion

A low serum level of vitamin B12 is associated with a severe decline in bone density in elderly Iranian women.

Conflict of Interest

The authors declare no conflict of interest in this study.

Acknowledgements

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References

1. Tucker KL, Hannan MT, Qiao N, Jacques PF, Selhub J, Cupples LA, et al. Low plasma vitamin B12 is associated with lower BMD: The Framingham Osteoporosis Study. *J Bone Miner Res*. 2005;20(1):152-8. doi: 10.1359/JBMR.041018. [PubMed: 15619681].
2. Saltzman JR, Russell RM. The aging gut. Nutritional issues. *Gastroenterol Clin North Am*. 1998;27(2):309-24. doi: 10.1016/s0889-8553(05)70005-4. [PubMed: 9650019].
3. Selhub J, Bagley LC, Miller J, Rosenberg IH. B vitamins, homocysteine, and neurocognitive function in the elderly. *Am J Clin Nutr*. 2000;71(2):614S-20S. doi: 10.1093/ajcn/71.2.614s. [PubMed: 10681269].
4. Baik HW, Russell RM. Vitamin B12 deficiency in the elderly. *Annu Rev Nutr*. 1999;19:357-77. doi: 10.1146/annurev.nutr.19.1.357. [PubMed: 10448529].
5. Wolters M, Strohle A, Hahn A. Cobalamin: A critical vitamin in the elderly. *Prev Med*. 2004;39(6):1256-66. doi: 10.1016/j.ypmed.2004.04.047. [PubMed: 15539065].
6. Brattstrom L, Wilcken DE. Homocysteine and cardiovascular disease: cause or effect? *Am J Clin Nutr*. 2000;72(2):315-23. doi: 10.1093/ajcn/72.2.315. [PubMed: 10919920].
7. Mangoni AA, Jackson SH. Homocysteine and cardiovascular disease: Current evidence and future prospects. *Am J Med*. 2002;112(7):556-65. doi: 10.1016/s0002-9343(02)01021-5. [PubMed: 12015248].
8. Nygard O, Vollset SE, Refsum H, Brattstrom L, Ueland PM. Total homocysteine and cardiovascular disease. *J Intern Med*. 1999;246(5):425-54. doi: 10.1046/j.1365-2796.1999.00512.x. [PubMed: 10583714].
9. Rosenberg IH. B vitamins, homocysteine, and neurocognitive function. *Nutr Rev*. 2001;59(8 Pt 2):S69-S73. doi: 10.1111/j.1753-4887.2001.tb05503.x. [PubMed: 11519670].
10. Carmel R. Current concepts in cobalamin deficiency. *Annu Rev Med*. 2000;51:357-75. doi: 10.1146/annurev.med.51.1.357. [PubMed: 10774470].
11. Holroyd C, Cooper C, Dennison E. Epidemiology of osteoporosis. *Best Pract Res Clin Endocrinol Metab*. 2008;22(5):671-85. doi: 10.1016/j.beem.2008.06.001. [PubMed: 19028351].
12. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. *Annu Rev Med*. 1998;49:31-62. doi: 10.1146/annurev.med.49.1.31. [PubMed: 9509248].
13. Golbahar J, Hamidi A, Aminzadeh MA, Omrani GR. Association of plasma folate, plasma total homocysteine, but not methylenetetrahydrofolate reductase C667T polymorphism, with bone mineral density in postmenopausal Iranian women: A cross-sectional study. *Bone*. 2004;35(3):760-5. doi: 10.1016/j.bone.2004.04.018. [PubMed: 15336613].
14. Morris MS, Jacques PF, Selhub J. Relation between homocysteine and B-vitamin status indicators and bone mineral density in older Americans. *Bone*. 2005;37(2):234-42. doi: 10.1016/j.bone.2005.04.017. [PubMed: 15950558].
15. Stone KL, Bauer DC, Sellmeyer D, Cummings SR. Low serum vitamin B-12 levels are associated with increased hip bone loss in older women: A prospective study. *J Clin Endocrinol Metab*. 2004;89(3):1217-21. doi: 10.1210/jc.2003-030074. [PubMed: 15001613].
16. Dhonukshe-Rutten RA, Lips M, de Jong N, Chin APM, Hiddink GJ, van Dusseldorp M, et al. Vitamin B-12 status is associated with bone mineral content and bone mineral density in frail elderly women but not in men. *J Nutr*. 2003;133(3):801-7. doi: 10.1093/jn/133.3.801. [PubMed: 12612156].
17. Cagnacci A, Baldassari F, Rivolta G, Arangino S, Volpe A. Relation of homocysteine, folate, and vitamin B12 to bone mineral density of postmenopausal women. *Bone*. 2003;33(6):956-9. doi: 10.1016/j.bone.2003.07.001. [PubMed: 14678855].
18. Sato Y, Honda Y, Iwamoto J, Kanoko T, Satoh K. Effect of folate and mecobalamin on hip fractures in patients with stroke: A randomized controlled trial. *JAMA*. 2005;293(9):1082-8. doi: 10.1001/jama.293.9.1082. [PubMed: 15741530].
19. McLean RR, Jacques PF, Selhub J, Fredman L, Tucker KL, Samelson EJ, et al. Plasma B vitamins, homocysteine, and their relation with bone loss and hip fracture in elderly men and women. *J Clin Endocrinol Metab*. 2008;93(6):2206-12. doi: 10.1210/jc.2007-2710. [PubMed: 18364381]. [PubMed Central: PMC2435634].
20. Fleurence RL. Cost-effectiveness of fracture prevention treatments in the elderly. *Int J Technol Assess Health Care*. 2004;20(2):184-91. doi: 10.1017/s0266462304000960. [PubMed: 15209178].
21. Vakili M, Forooghan M, Nojomi M, Ghaleh Bandi MF, Khodabandeloo N. A survey on serum levels of B12, folate and homocysteine in healthy elderly Tehranis. *Razi J Med Sci*. 2009;16(63):189-200. [In Persian].
22. Hong X, Hsu YH, Terwedow H, Tang G, Liu X, Jiang S, et al.

Association of the methylenetetrahydrofolate reductase C677T polymorphism and fracture risk in Chinese postmenopausal women. *Bone*. 2007;40(3):737-42. doi: 10.1016/j.bone.2006.09.031. [PubMed: 17174622]. [PubMed Central: PMC1855293].
23. Yilmaz N, Kepkep N, Cicek HK, Celik A, Meram I. Relation of parity and homocysteine to bone mineral density of postmenopausal women. *Clin Lab*. 2006;52(1-2):49-56. [PubMed: 16506364].
24. Zhang H, Tao X, Wu J. Association of homocysteine, vitamin B12, and folate with bone mineral density in postmenopausal women: A meta-analysis. *Arch Gynecol Obstet*. 2014;289(5):1003-9. doi: 10.1007/s00404-013-3075-6. [PubMed: 24193243].
25. Haliloglu B, Aksungar FB, Ilter E, Peker H, Akin FT, Mutlu N, et al. Relationship between bone mineral density, bone turnover markers and homocysteine, folate and vitamin B12 levels in postmenopausal women. *Arch Gynecol Obstet*. 2010;281(4):663-8. doi: 10.1007/s00404-009-1297-4. [PubMed: 19946695].
26. Bozkurt N, Erdem M, Yilmaz E, Erdem A, Biri A, Kubatova A, et al. The relationship of homocyteine, B12 and folic acid with the bone mineral density of the femur and lumbar spine in Turkish postmenopausal women. *Arch Gynecol Obstet*. 2009;280(3):381-7. doi: 10.1007/s00404-009-0936-0. [PubMed: 19151987].
27. You L, Sheng Z, Zhang L, Chen J. Relation of serum vitamin B12 and serum folate to bone mineral density in postmenopansal women. *Chinese Journal of Osteoporosis*. 2007;13(1):26-8.
28. Selhub J. The many facets of hyperhomocysteinemia: studies from the Framingham cohorts. *J Nutr*. 2006;136(6 Suppl):1726S-30S. doi: 10.1093/jn/136.6.1726S. [PubMed: 16702347].
29. Herrmann M, Kraenzlin M, Pape G, Sand-Hill M, Herrmann W. Relation between homocysteine and biochemical bone turnover markers and bone mineral density in peri- and post-menopausal women. *Clin Chem Lab Med*. 2005;43(10):1118-23. doi: 10.1515/CCLM.2005.195. [PubMed: 16197308].