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Original Article

Association of Bone Mineral Density with Vitamin B12 Levels in Patients Aged 65 Years and Over☆

Ş.H. Aktaş *, S. Ucak

Internal medicine Clinic, Umraniye Research and Training Hospital, Istanbul, Turkey

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SUMMARY

Background: With the ageing world population, osteoporosis becomes a major health issue. Vitamin and mineral deficiencies are the preventable causes of osteoporosis; and data regarding the effects of vit B12 deficiency on bone mineral density (BMD) remain conflicting. Consequently, we aimed to investigate the effects of vit B12 deficiency, which is common in patients older than 65 years of age, on osteoporosis.
Methods: This cross-sectional study was performed on 118 patients aged 65 and over who were admitted to the internal medicine outpatient clinics of the hospital. Patients were divided into 3 groups based on their vit B12 levels: vit-B12 levels lower than 200 pg/mL (group 1), between 200 and 300 pg/mL (group 2) and over 300 pg/mL (group 3). BMDs obtained from the total hip, femoral neck and lumbar spine regions were compared between groups. Serum Ca, folate, thyroid stimulating hormone (TSH) and free T4 (FT4) levels were also compared between groups.
Results: There was a statistically significant difference between groups regarding femur neck BMD ($p < 0.001$).
Conclusion: We found that the bone mineral density at the femur neck was lower when we were below vit B12 level 300 pg/ml. We recommend that bone mineral density must be measured in elderly people who are found to have vit B12 deficiency.

1. Introduction

Osteoporosis is one of the most important problems of the ageing world population because it not only causes mortality and morbidity. Osteoporosis reduces the health quality and sources an important financial problem in health burden. The treatment and maintenance of fractures associated with the fractures cause an increased burden to the individuals and also to the social security departments and economy of the countries. More than 200 million people in the world today are estimated to be osteoporotic.

Bone fractures that develop due to osteoporosis, particularly hip fractures, decrease the life quality of life and life expectancy of patients because they force the patient to be dependent on someone else and complications associated with osteoporotic fractures can lead to excess mortality. Approximately 15%–20% of older patients, die owing to femoral fractures annually and approximately the half of the affected patients become dependent on caregiver. Moreover, each fracture is a forerunner of another fracture that may occur in another parts of the body.

In the year 2000, approximately 9 million fractures occurred worldwide, leading to a loss of 5.8 million disability adjusted life-years (DALYs).[1] Regarding the prevalence of osteoporosis in Europe, it is estimated that by the year 2050, the number of men and women to be affected will be more than 30 millions. Due to a rise in life expectancy, the economic burden of osteoporotic fractures in Europe is expected to increase substantially in the coming decades. Osteoporotic subjects represent a group of individuals at risk, thus scientists and clinicians are interested in identifying specific nutrients that can ensure high-quality bone health and prevent disease-related skeletal complications.[2]

Osteoporosis is a complex, multi-factorial disease with a strong genetic component; however, lifestyle factors also have significant effects on bone mass. Nourishment is one of the most important lifestyle factors affecting bone health. In the first decades of life, a sufficient amount of calcium (Ca) and vitamin D should be consumed to compose peak bone mass. Similar to nutritional deficiencies causing growth retardation and skeletal system deformities in childhood; inadequate consumption of nutrients such as proteins, vitamins and minerals can result in decreased in bone quality in adults.

In the elderly, nutritional deficiencies are very common and adequate nutritional intake may slow down related loss of bone mass.[3] Due to both a decrease in consumed nutrients and malabsorption, vitamin deficiencies are common in the elderly population. With advanced age, gastric emptying time is delayed, hormonal responses are disturbed, basal metabolism rate slows down, and taste and odour sensations are affected, resulting in a decrease in sufficient daily intake of calories, protein, minerals and vitamins.

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* Corresponding author.
E-mail addresses: drhsaktas@gmail.com (Ş.H. Aktaş).

Vitamin B12 (vit-B12) deficiency is reported at a rate ranging between 5 and 20% among the elderly. Morever, has a wide range of symptoms including pain, paresthesia, loss of temperature or touch sensations, chilling, tiredness, visual disturbances, incontinence, confusion, loss of memory, apathy, irritability, personality changes, depression, hallucinations, tachycardia, loss of appetite, alterations in blood pressure, tinnitus and premature grey hair. The negative effects of vit B12 deficiency on hematopoietic and neurological systems are well documented. Moreover, it also acts in cardiovascular and cerebrovascular accidents.[4] However, studies exploring its effects on the skeletal system are still ongoing.[5]

In recent years, vit B12 has gained attention due to the many epidemiological studies reporting the association of vit-B12 levels with bone quality and fracture risk.[6]

With a decrease in vit-B12 intake in the elderly, in addition to a decrease in serum vit-B12 levels, moderate hyperhomocysteinemia increasing with age as well as elevated serum methylmalonic acid (MMA) levels occur. Several studies have reported that low plasma B12 levels are associated with reduced bone quality.[7] However, other studies have not observed this association.[8] Some observations suggest that increased homocysteine (Hcy) is a predictive factor for fracture risk.[9] Also, elevated MMA levels in the elderly and low bone mineral density (BMD) in adolescent are associated with osteoporosis. Together, these data suggest that vit B12, Hcy, and MMA are important factors in the development of osteoporosis.

With the ageing world population, osteoporosis becomes a major health issue. Vitamin and mineral deficiencies are the preventable causes of osteoporosis; and data regarding the effects of vit B12 deficiency on BMD remain conflicting. Consequently, we aimed to investigate the effects of vit B12 deficiency, which is common in patients older than 65 years of age, on osteoporosis.

2. Patients and methods

2.1. Design and patients

This cross-sectional study was performed on 118 patients aged 65 and over who were admitted to the Internal Medicine outpatient clinics of the hospital. The research protocol was in accordance with the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of Umraniye Education Research Hospital. (B.10.1.TKH.4.34.H.GP.0,01/58). All subjects provided written informed consent before inclusion in the study.

Exclusion criteria were presence of malignancies, endocrine disease (diabetes mellitus, thyroid disease, hyperparathyroidism, etc), malabsorption, chronic liver disease, patients with vitamin D deficiency, chronic renal disease, use of any type of drugs affecting BMD (glucocorticoids, thiazide diuretics, anticoagulants, anticonvulsants, estrogens etc) and patients undergoing treatment for osteoporosis (biphosfonates, vit-D or Ca).

Vit B12 levels of patients included in the study were evaluated as;

Over 300 pg/ml: normal,
200–300 pg/ml: borderline,
Lower than 200 pg/ml: low.[10]

Patients were divided into 3 groups based on their vit B12 levels: vit-B12 levels lower than 200 pg/mL (group 1), between 200 and 300 pg/mL (group 2) and over 300 pg/mL (group 3). BMDs obtained from the total hip, femoral neck and lumbar spine regions were compared between groups. Serum Ca, folate, thyroid stimulating hormone (TSH) and free T4 (FT4) levels were also compared between groups.

BMDs of the total hip, femoral neck and lumbar spine (L1-L4) regions of study participants were obtained using dual – energy X-ray absorptiometry (DXA) (Horizon Wi, S/N 200894 model).

Vit –B12, folic acid, TSH and FT4 levels were obtained with the chemiluminescence method (Abbott ARCHİTECT i2000), whereas Ca was measured using the calorimetric method (Abbott Clinical Chemistry ARCHİTECT 16000).

2.2. Statistical analysis

Descriptive statistics were performed to define the continuous variables (mean, standard deviation, and minimum, median and maximum). The association of more than 2 independent variables with normal distribution was investigated using one way analysis of variances. The association of more than 2 independent variables with non-normal distribution was investigated using the Kruskal Wallis test. Post-Hoc binary evaluations between groups were performed using Bonferroni corrected Mann Whitney *U* test. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013).

3. Results

In total 118 patients included in the study were categorized based on their vit B12 levels as group 1–(low) (n = 40), group 2-(borderline) (n = 38) and group 3-(normal) (n = 40). Mean age of the study participants was 72.9 ± 6.8 years. 28.8% were male and 71.2% were female. Mean BMI of participants was 29.79 ± 5.92 kg/m^2 (Table 1).

There was not any statistically significant difference between groups regarding age, gender or BMI. Likewise no statistically significant difference was found between groups regarding TSH, FT4, Ca, Folate levels or between groups regarding T score, and total hip (BMD) results. Although there was a statistically significant difference between groups regarding L1-L4 BMD results, there was no significant difference when the groups were compared with each other. There was a statistically significant difference between groups regarding femur neck BMD results ($p < 0.001$) (Table 2).

There was a statistically significant difference between groups 1 and 3 regarding femur neck BMD results (Bonferroni correction was performed and p value lower than 0.0166 was regarded as statistically significant). There was also a statistically significant difference between groups 2 and 3 regarding femur neck BMD results but not between groups 1 and 2 regarding femur neck BMD results (Table 3).

4. Discussion

Osteoporosis is a progressive metabolic bone disease involving

Table 1
Demographic data.

	N	Mean	Median	SD	Min.	Max.
Age (years)	118	72.9	71	6.8	61	92
BMI	118	29.79	29	5.92	17	44
Gender distribution			N			%
Male			34			28.8
Female			84			71.2
Total			118			100

low bone mass and disturbed micro-architecture of bone tissue with a resulting increase in bone fragility and fracture tendency. Osteoporosis and associated bone fracture risk increases with advancing ages. Moreover, gender, ethnicity, physical activity, drinking, smoking, and other factors such as estrogen, Ca and vitamin D levels are also related to osteoporosis. B complex vitamins (folate, B12 and B6) affect bone health individually as well as total plasma homocysteine concentrations.[11]

Until now, the nutrients most important in osteoporosis prevention have been Ca and vitamin D. However, in recent years, it has been determined that other nutritional factors are also required for. Although more studies are warranted, sufficient intake of B complex vitamins, in particular, is considered to restore bone health.[12]

In this study, in patients aged 65 or over, if Vit B12 levels are lower than the normal (300 pg/ml), femur neck bone mineral density was determined to be lower. However we did not determine any differences between the patients with low Vit B12 levels (<200 pg/ml) and borderline Vit B12 levels (200–300 pg/ml). Furthermore, if vit B12 levels are lower than 300 pg/ml; femur neck BMD decreases and we suggest that this augments the risk of osteoporosis and hip fracture.

In literature, similar with our results, there are some studies investigating the association of vit B12 deficiency with the osteoporosis. Tucker et al found, a correlation between the vit B12 deficiency and BMD decline in the lumbar region. Moreover, in the same study, bone fractures were determined to increase with vit B12 deficiency in both men and women.[6]

In a study of Bozkurt et al there was an association plasma Hcy levels and osteoporosis in postmenopausal Turkýsh women.[13] In a large cohort study on post-menopausal women performed by Bucciarelli et al, a negative association between total femur BMD and Hcy levels was reported.[14] Ouzzif et al described vit-B12 and Hcy levels as a risk factor for osteoporosis in Moroccan postmenopausal women.[15] Zhu et al determined that high Hcy levels were associated with greater hip bone loss in geriatric women with 70–85 years of age.[16] In a study of Bahtiri et al on post-menopausal women high Hcy levels were defined as an independent risk factor for osteoporosis.[17] El Maghraoui et al reported that in elderly women with low serum vit-B12 levels total femur BMD loss was greater.[18] In a study of Naharci et al protective effects of normal vit-B12 levels on femur neck BMD was defined.[19]

In the Framingham Osteoporosis Study, patients with vit B12 levels lower than 148 pmol/L, BMD was determined as worse. This difference was reported especially on hip in males and in the spine in females.[6]

In the Hordaland Homocysteine Study, elevated plasma total homocysteine (tHcy) levels and low foliate levels were found to be associated with low BMD results in women. Moreover tHcy was emphasised as a potential modifiable risk factor of osteoporosis in women.[9]

Unlike all of those studies, there are also some studies in literature reporting that vit –B12 levels do not have any effects on BMD. In a study of Kakehasi et al on post-menopausal Brazilian women it was determined that there was not any association between Vit –B12 deficiency and BMD.[20] Cagnacci et al and Rumbak et al also reported association between vit-B12 levels and BMD.[17] In a study of Haliloğlu et al on post-menopausal women, there was not any association determined between serum Hcy and vit –B12 levels and lumbar spine BMD.[21] In a study of Herrmann et al on rats foliate and vit –B12 deficiencies were reported not to have any effects on bone health.[22]

When all of the previous studies are investigated carefully, the answer of the question 'How do B vitamins affect the bone health?' focuses on the Hcy. Vit –B12 is an important cofactor in homocysteine metabolism and in conditions with Vit –B12 deficiency, hyper-homocysteinemia is seen. The pathophysiology of bone fractures associated with the hyper-homocysteinemia is still not

Table 3
Comparison of L1-L4 and Femur neck BMD results between groups according to vitamin B12 levels with Post-Hoc analysis.

	L1-L4 p[a]	Femur neck p[b]
Group 1 – Group 2	0,068	0841
Group 1 – Group 3	1,00	**<0,001**
Group 2 – Group 3	0,072	**<0,001**

Bold indicates p< 0.05.
[a] Mann Whitney U test.
[b] Duncan Test.

Table 2
Comparison of parameters according to vitamin B12 levels.

	Group 1(vit B12 < 200) n = 40		Group 2(vit B12 200–300) n = 38		Group 3(vit B12 > 300) n = 40		P
	Mean ± SD	Med (min-max)	Mean ± SD	Med (min-max)	Mean ± SD	Med (min-max)	
Age	72,9 ± 7,8	71 (63–92)	71,5 ± 6,1	70 (61–88)	74,4 ± 6,2	74 (65–90)	0,066[b]
BMI	29,1 ± 5,9	28,5 (19–44)	30,8 ± 6,4	31 (17–44)	29,6 ± 5,4	29,5 (18–40)	0,428[a]
Gender N(%)							
Male	13 (32,5)		9 (23,7)		12 (30)		
Female	27 (67,5)		29 (76,3)		28 (70)		0,677[c]
TSH	1,8 ± 1,5	1,6 (0,1–7,3)	1,9 ± 1,7	1,4 (0,4–8,7)	2,1 ± 1,8	1,7 (0,1–9,7)	0,707[b]
FT4	0,9 ± 0,2	0,9 (0,0–1,8)	0,9 ± 0,2	1,0 (0,1–1,3)	1,0 ± 0,2	1,0 (0,5–1,3)	0,551[b]
Foliate	7,1 ± 3,1	7,2 (2–13)	6,2 ± 2,1	6,0 (3–10)	7,2 ± 3,2	6,9 (2–15)	0,210[a]
Ca	9,3 ± 0,6	9,3 (8–11)	9,3 ± 0,4	9,3 (8–10)	9,2 ± 0,6	9,2 (7–10)	0,778[a]
T Score L1-L4	–1,9 ± 1,6	–2,2 (–4,8–2)	2,4 ± 1,0	–2,5 (–4,9 (–0,3))	1,8 ± 1,4	–1,9 (–4,3–1,3)	0,131[a]
L1-L4 BMD	0,9 ± 0,2	0,8 (0,6–1,3)	0,8 ± 0,1	0,8 (0,5–1,1)	0,9 ± 0,2	0,9 (0,5–1,2)	**0,040**[a]
Femur neckBMD	0,6 ± 0,1	0,6 (0,30,9)	0,6 ± 0,1	0,6 (0,3–0,8)	0,8 ± 0,3	0,8 (0,42,0)	**<0,001**[b]
Total hip BMD	0,8 ± 0,2	0,8 (0,5–1,3)	0,8 ± 0,1	0,8 (0,5–1,1)	0,8 ± 0,2	0,8 (0,6–1,2)	0,530[b]

Bold indicates p< 0.05.
[a] One Way ANOVA.
[b] Kruskal Wallis test.
[c] Chi -Squared.

clearly elucidated yet. In some studies, the effects of Vit B12 and homocysteine on osteoblasts and osteoclasts are emphasized. Moreover, they may also have some effects on BMD, bone turnover, bone blood flow and collagen cross links. Elevated Hcy levels may cause a decrease in BMD, alterations in micro-architecture and an increase in bone fragility.[23]

In in vitro studies have reported that osteoclastic activity and bone resorption increase with an increase in serum Hcy concentrations (from 10 mmol L-1), thus defining the inhibitory effects of Hcy on bone formation.[23]

In a study of Kang and Trelstad, it was determined that with the collagen cross links purified from the rat skin collagen was interacting with homocysteine. In some other in vitro studies, high Hcy concentrations were reported to inhibit the lysyl oxidase (an enzyme involved in cross-linking of collagen) activity and by this way in high concentrations it was determined to stimulate the osteoclastic activity. In another in vitro study, the interaction of Hcy with collagen cross links was shown to cause alterations in bone matrix resulting in a more fragile bony structure. In experimental studies, folic acid, vit B6 and vit B12 deficiencies were reported to cause an increase in Hcy levels which results in free radical formation and augmented oxidative stress causing endothelial dysfunction and consequently diminished bony blood flow and at the end osteoporosis.[23]

Clement et al observed that vit-B12 synthesis deficit mice were having growth retardation and they were poor for osteoblasts. Evidence emerging in vivo suggests that vit B12 may interfere with growth hormone signaling in these mice and exert its downstream effects on osteoblasts.[23]

In a study investigating the effects of vit-B12 on osteoblast associated proteins, in patients with vit-B12 deficiency, serum osteocalcin and skeletal alkaline phosphatase levels were determined to be lower. These data showed that, osteoblastic activity depends on the vit-B12 and bone metabolism is affected from the vit-B12 deficiency.[23]

B.L.T.Vaes et al investigated the effects of vit-B12, Hcy and MMA levels on differentiation of mesenchymal stem cells to the osteoblasts and osteoclasts in bone marrow cultures in their study. They determined that vit-B12 deficiency, by increasing MMA and Hcy levels, was increasing osteoclast formation and thus lessening bone mass.[3]

Homocysteine is a sulphur containing amino acid produced during methionine metabolism.[24] It is metabolized by *trans*-sulphuration or re-methylation cascades. In re-methylation cascade, vit-B12 takes place. Homocysteine levels are affected from genetic defects in metabolism (such as enzyme defects), chronic diseases, vitamin or nutritional defects, individual features (age, gender and etc) and some drugs.

Homocysteine levels especially increase with age, and they are determined to be higher in males compared with the females. Elevated plasma homocysteine levels causes hyperhomocysteinemia and concomitant homocictinuria. Elevated plasma Hcy level is an important risk factor for many diseases including arterial and venous thrombosis, stroke, myocardial infarction, and chronic renal failure. Elevated Hcy levels may be normalized with folic acid consumption.

It was suggested that, homocysteine disturbs collagen crosslinks and this may cause osteoporosis by disconcerting mineralization and matrix structure. Moreover, hyper-homocysteinemia was suggested to increase the osteoclasts while not affecting the osteoblasts.[25]

The aim of the randomized, controlled the B-PROOF study (B vitamins for the Prevention of Osteoporotic Fracture) was investigating if vit-B12 and folic acid supplementations decrease the bony fractures or not in elderly patients with hyper-homocysteinemia. However, at the end of the study, in elder population, vit-B12 and folic acid supplementations were reported not to have any effects on osteoporotic fractures. Since this treatment was associated with higher cancer incidence, in elderly population, in prevention of fractures, vitamin B12 plus folic acid supplementation is not advised.[26]

In conclusion, we determined that the bone mineral density at the femur neck was lower when we were below vit B12 level 300 pg/ml. It is still controversial whether vit-B12 deficiency causes osteoporosis and new studies on this topic are warrented. However, with an increase in ageing population in all over the world, osteoporosis becomes an increasingly important health problem. The treatment and maintenance of fractures associated with the fractures cause an increased burden to the individuals and also to the social security departments and economy of the countries. However, the treatment of vit-B12 deficiency is highly cheap and easy. For that reason, in elderly patients, we believe that, vit-B12 deficiency should be investigated and if present should be treated. We recommend that bone mineral density must be measured in elderly people who are found to have vit B12 deficiency.

Conflict of interest

None.

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