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Original Research Article

Association of Vitamin B12 with Bone Mineral Density in Postmenopausal Women in Bangladesh

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Abstract

The risk of osteoporosis is higher in postmenopausal women and nutritional factors reported playing an important role in the etiology of low bone mineral density (BMD). Some studies claimed the involvement of vitamin B_{12} in the quality of bone structure in humans, however, results are not conclusive. The objective of this study was to explore the relationship between BMD and vitamin B_{12} levels in postmenopausal women. In this cross-sectional study, BMD and blood samples from 77 postmenopausal women (age > 45 years) were studied. BMD and T-scores of the study subjects were determined at the femoral neck and lumbar spines by Dual Energy X-ray Absorptiometry (DEXA). Serum $VitB_{12}$ was measured by enzyme-linked immunosorbent assay. The mean±SD age of the postmenopausal women was 56.4±7.9 years. Bone mineral densities (g/cm^2) were 0.80±0.16, 0.76±0.18, 0.74±0.14 and T-scores were -2.21±1.45, -1.41±1.22, -1.53±1.20 respectively at the lumbar spine, right femoral neck and left femoral neck. The mean±SD of $vitB_{12}$ was 245.9±274.3 pg/mL. On multiple regression analysis, β values for $\log(VitB_{12})$ with BMD were 0.119 (p = 0.018), 0.085 (p = 0.140), 0.011 (p = 0.012) and with T-score were 1.028 (p = 0.022), 0.698 (p = 0.064), 0.940 (p = 0.015) at the lumbar spine and right femoral neck and left femoral neck respectively. In conclusion, vitamin B_{12} is found to be positively associated with bone mineral density and T-score at the lumbar spine and left femoral neck but not at the right femoral neck in postmenopausal women.

Keywords: Bone Mineral Density, Vitamin B12, Osteoporosis.

INTRODUCTION

Osteoporosis is a chronic, progressive but silent disorder characterized by a decrease in bone mineral density (BMD) that reflects the calcium and phosphorus concentrations in a particular volume of bone and deterioration of the skeletal microarchitecture, leads to fragility and increased susceptibility to bone fractures (Kakehasi *et al*., 2012; Lee *et al*., 2010; Kanis *et al*., 1994). Low BMD is frequently observed in elderly women (Lim *et al*., 2005) and it is most prevalent in postmenopausal women (Bock *et al*., 2008). Osteoporosis was found in 41.8% of elderly (age above 45 years) Bangladeshi women (Begum *et al*., 2014) and a higher proportion (49.6%) was observed in postmenopausal women (Chowdhury *et al*., 2001; Ahmed *et al*., 2019). In postmenopausal women, reduced estrogen prevents the absorption and utilization of calcium which facilitates the development of osteoporosis in older women (Riggs *et al*., 1986). Osteoporosis weakens the bones and leads to fractures. Patients with osteoporosis experienced long-term morbidity including chronic pain, deformity, and disability, which indirectly give rise to loss of normal functioning. It is considered one of the most important health problems that pose a negative impact on society.

Many factors are associated with the etiology of low BMD; some of them are nutritional origin (Ahmed *et al*., 2019; Riggs *et al*., 1986; Ooms *et al*., 1995; Napoli *et al*., 2014; Van Wijngaarden, 2011). The impact of vitamin D, calcium, phosphorous and parathyroid hormone (Lips and van Schoor, 2011; Sahota, 2000) is well known. However, some studies demonstrated the beneficial role of vitamin B_{12} in the quality of bone structure in humans in different populations or clinical interventions (McLean *et al*., 2008; Roman-Garcia *et al*., 2014; Dai *et al*., 2015; Melton *et al*., 1994). In Framingham Osteoporosis longitudinal follow-up study (England), vitamin B_{12} was found to be inversely associated with hip fracture risk (McLean *et al*., 2008). Vitamin B_{12} deficiency in a

murine genetic model, decreases liver taurine and oral supplementation of taurine enhanced osteoblast functions and subsequently reduced osteoporosis (Roman-Garcia *et al.*, 2014). Another 2-year follow-up study demonstrated that osteoporosis associated with pernicious anemia was markedly improved by vitamin B_{12} supplement therapy (Melton *et al*., 1994). The formation of collagen or alteration to the metabolism of osteoblasts was found to be modulated by Vitamin B_{12} in a dose-dependent manner (Kim *et al*., 1996; Bailey *et al*., 2015). The deficiency of vitamin B12 was found to increase the risk of low BMD and fractures (Clarke *et al*., 2015). Osteocalcin secreted by osteoblast, one of the markers of bone formation (Marrone *et al*., 2012; Wheater *et al*., 2013), was found to be higher in premenopausal women with low BMD compared to normal BMD (Jeong *et al*., 2014). In a cross-sectional observational study in Turkish postmenopausal women with low BMD in the femoral neck and in the vertebrae was found to have significantly lower serum levels of vitamin B_{12} (Bozkurt *et al*., 2009). On the other hand, a study consisting of 328 British postmenopausal women, found no significant association between BMD and vitamin B_{12} (Baines *et al*., 2007). In another cross-sectional observational study consisting of 131 Croatian women also revealed a lack of association between BMD and vitamin B_{12} (Rumbak *et al*., 2012). Thus, the relationship between vitamin B_{12} and BMD was found to be varied in different populations. In our population, it remains to be evaluated. By considering the worldwide rise in fracture due to osteoporosis and population exposed in developing countries, particularly in postmenopausal women in Bangladesh (Jabin *et al*., 2017), this study aimed to evaluate the role of vitamin B_{12} on bone mineral density.

MATERIALS AND METHODS

Study design and sample

Seventy-seven (77) postmenopausal women (age> 35 years) without a history of known comorbid diseases, were included in this cross-sectional study and was conducted in the Dept. of Applied Laboratory Sciences, Bangladesh University of Health Sciences (BUHS), Dhaka, Bangladesh, referred for screening for osteopenia or osteoporosis to the National Institute of Nuclear Medicine & Allied Sciences, Bangladesh Atomic Energy Commission. Volunteers were informed in detail about the purpose and nature of the study and written consent was obtained from willing subjects. History of diabetes mellitus and hypertension and the Body mass index (BMI) of the study subjects were recorded.

Determination of Bone mineral density and serum vitB_{12}

Bone mineral density was scanned at the lumbar spine (L2 to L4) and proximal femur by dual-energy X-ray absorptiometry (DEXA) (Muraduzzaman *et al*., 2021) using a GE Lunar DPX-NT PRO (Lunar Corp, Adison, WI, USA) in the National Institute of Nuclear Medicine & Allied Sciences, Bangladesh Atomic Energy Commission, Dhaka, Bangladesh, following standard protocols and expressed in g/cm^2 and as peak bone mass percentage (T-score) against the normal subjects. Levels of serum Vit B_{12} were measured by enzyme-linked immunosorbent assay (Assaypro, USA).

STATISTICAL ANALYSIS

The distribution and skewness of the data for continuous variables were tested, and were presented as mean ± standard deviation. Data that rejected normal distribution were logtransformed for correlation and multiple regression analysis using MedCalc version 11.4 and a p-value < 0.05 was considered as significant.

RESULTS

Characteristics of the study subjects

The age range of the study subjects was 35 to 75 years. Characteristics of the study subjects are shown in Table 1. Of the 77 subjects, 42 (54.5%) were hypertensive and 35 (45.5%) were normotensive; 21 (27.3%) were diabetic, 56 (72.7%) were nondiabetic, 29 (37.7%) were overweight (BMI: 25-30 kg/m^2) and 11 (14.3%) were obese (BMI>30 kg/m^2). The age distributions followed a normal distribution (p = 0.196) (Fig 2).

Table-1: Characteristics of the study subjects

Variable	Mean±SD/%
Age (years)	56.4±7.9
BMI (kg/m^2)	25.8±4.2
Hypertension	54.5%
Systolic blood pressure (mmHg)	126±17
Diastolic blood pressure (mmHg)	81±12
Diabetes	27.3%

Results were expressed as mean±SD/%; BMI, Body mass index

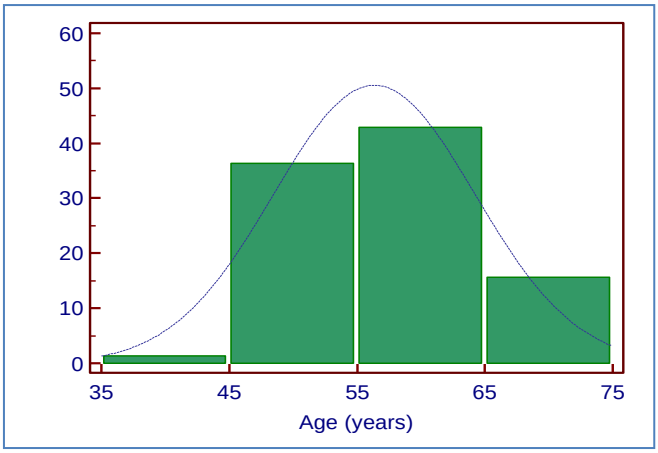

Fig-2: Age distribution of the study subjects

Bone mineral density in the study subjects

Bone mineral density BMD at the lumbar spine was significantly higher than right femoral neck (p = 0.006) and left femoral neck (p <0.001) but similar at the right and the left femoral neck (p = 0.246). Similarly, T-score was higher at the lumbar spine than the femoral neck (p < 0.001) and similar in the femoral necks (p = 0.243) (Table 2).

Table-2: BMD in the lumbar spine and the femoral neck

Location	BMD (g/cm^2)	T-score
Lumbar spine	0.80±0.16	-2.21±1.45
Right femoral neck	0.76±0.18	-1.41±1.22
Left femoral neck	0.74±0.14	-1.53±1.20

Vitamin B_{12} status of the study subjects

The mean±SD of vitB_{12} was 245.9±274.3 pg/mL. The distribution rejects normality (p<0.001). The range of vitB_{12} was 47.1 to 1846.3 pg/mL. Fig 3 represented the Box-and-Whisker plot of VitB_{12} values after logarithmic transformation.

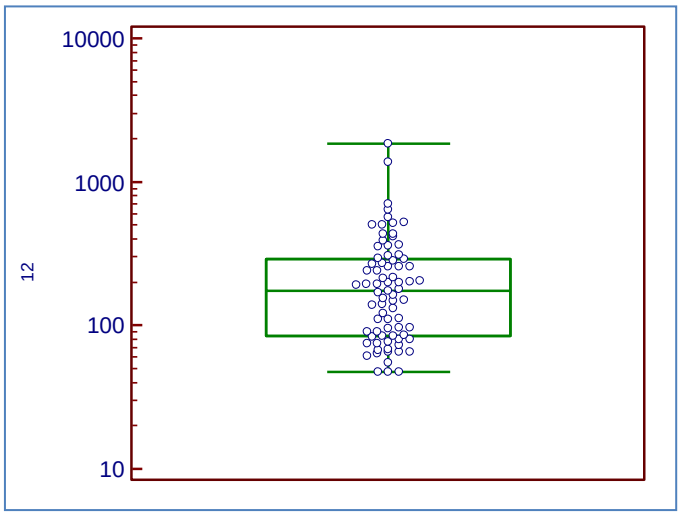

Fig-3: Box-and-Whisker plot of vitB_{12}

Relationship of BMD and T-score with VitB_{12} in postmenopausal women

The correlation coefficients of BMD and T-score at the lumbar spine, right femoral neck and left femoral neck are presented in Fig 4 and Fig 5. BMD and T-scores at the lumbar spine and left femoral neck was significantly related to log(vitB_{12}) (Fig 4A, Fig 4C and Fig 5A, Fig 5C). The correlation coefficients of BMD and T-score with log(vitB_{12}) were not statistically significant (Fig 4B and Fig 5B).

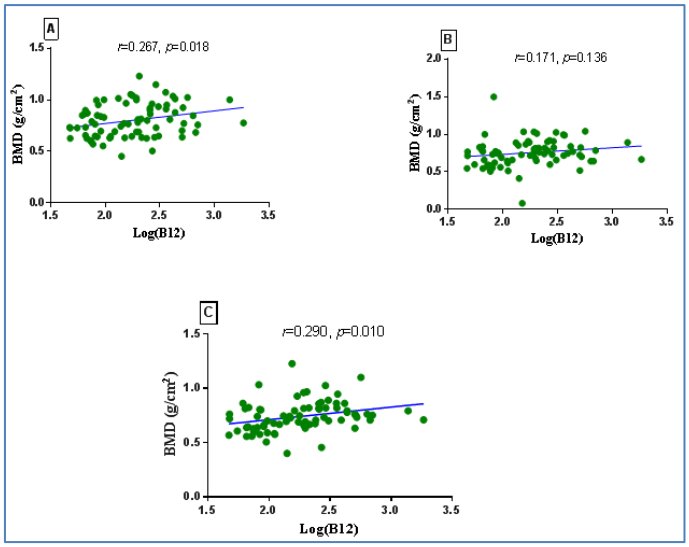

Fig-4: Correlation of BMD with VitB12 at the lumbar spine (A), right femoral neck (B) and left femoral neck (C)

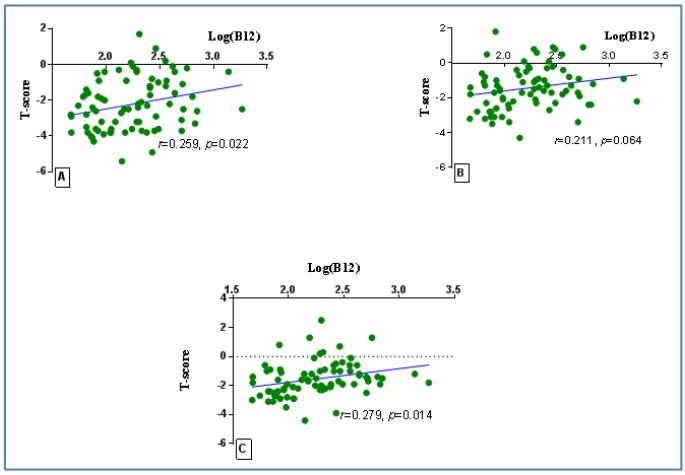

Fig-5: Correlation of T-score with VitB_{12} at the lumbar spine (A), right femoral neck (B) and left femoral neck (C).

Multiple regression analysis

The coefficient of BMD at the lumbar spine, right femoral neck or left femoral neck in multiple regression analysis considering age, BMI, Log(vitB_{12}), hypertension and diabetes, as independent variables presented in Table 3. BMD and T-scores at the lumbar spine and left femoral neck showed a significant positive association with log(vitB_{12}) (Table 3) but BMD and T-score at right femoral neck showed no such association with log(vitB_{12}) on adjusting confounding variables (Table 3).

Table-3: Coefficient of BMD in multiple regression analysis

	Lumbar spine		**Right femoral neck**		**Left femoral neck**	
BMD (g/cm^2)	β	p-value	β	p-value	β	p-value
Age	-0.003	0.195	-0.006	0.039	-0.001	0.449
BMI	0.012	0.006	0.009	0.073	0.013	0.009
LogB_{12}	0.119	**0.018**	0.085	0.140	0.110	**0.012**
HTN	-0.059	0.129	-0.061	0.173	-0.063	0.062
DM	-0.005	0.903	0.014	0.761	0.004	0.911
T-score						
Age	-0.028	0.176	-0.031	0.071	-0.011	0.535
BMI	0.107	0.007	0.083	0.013	0.084	0.013
LogB_{12}	1.028	**0.022**	0.698	0.064	0.940	**0.015**
HTN	-0.535	0.121	-0.504	0.084	-0.378	0.202
DM	-0.062	0.864	-0.064	0.835	0.211	0.497

BMI, body mass index; HTN, hypertension and DM, diabetes mellitus

DISCUSSION

Osteoporosis is a major health problem among postmenopausal women (Bock *et al*., 2008; Ahmed *et al*., 2019). Though estrogen (Riggs *et al.*, 1998) and Vitamin D (Sahota, 2000; Lips and van Schoor, 2011); deficiency play key roles, Vitamin B_{12} (vitB_{12}) deficiency may also facilitate the process (Kim *et al*., 1996; Bailey *et al*., 2015; Dai *et al*., 2015). In this cross-sectional study, the relationship between osteoporosis and vitB_{12} was evaluated in a group of postmenopausal women of Bangladeshi Nationality.

In this study, a positive relationship was found between serum vitB_{12} levels and bone mineral density (BMD) and T-score at the lumbar spine and left femoral neck. On multiple regression analysis, an independent positive association was found between vitB_{12} and BMD and T-score measured at the lumbar spine and left femoral neck but not with BMD and T-score measured at the right femoral neck after adjusting the confounding variables. This finding is consistent with findings of the Framingham osteoporosis study done on the American population (Tucker *et al*., 2005) where decreased vitB_{12} was shown to be associated with low bone mass in hip and lumbar spine, and suggested that vitB_{12} may be a controllable risk factor for prevention of osteoporosis. In another study done on the American population, Morris *et al*. reported that indicators of vitB_{12} status (serum vitB_{12} level and methyl malonic acid) were positively associated with BMD in elderly (Morris *et al*., 2005). McLean *et al*., in a study found that BMD is lower in people with decreased vitB_{12} (McLean *et al*., 2008) which increases the risk of fracture. In another study done by Bozcurt et al found a significant association of low BMD and low level of vitB_{12} in postmenopausal women at the femoral neck and lumbar spine which is consistent with our findings (Bozcurt *et al*., 2009).

On the other hand, Rejnmark *et al*. found no association between BMD and vitB_{12} in a cross-sectional study but found a positive association between BMD and dietary Folate (Rejnmark *et al*., 2008). In another study performed by Holstein *et al*. found no significant association of trabecular bone mass with serum level of vitB_{12} on the patient with osteoarthritis (Holstein *et al*., 2009). Rumbak *et al*. also found no significant association between BMD and serum level of vit B_{12} (Rumbak *et al*., 2012). A study performed to find an association between vit B_{12} (Dietary and serum levels) and BMD in patient with celiac disease found a positive association BMD and Vit B12 among men but not in women (Clarke *et al*., 2015). Bailey *et al*. found no direct association of vit B_{12} with BMD but the main indicator of this vitamin, and methyl malonic acid (MMA) and serum Homocysteine (Hcy) levels were associated significantly with BMD (Bailey *et al*., 2015). A similar result was observed by Cagnacci *et al*. (2003, 2008) and Gjesdal *et al*. (2006). Yazdanpanah *et al*. (2007), Rejnamark *et al*. (2008) or Dai *et al*. (2013) found no significant association between dietary intake of vit B_{12} and BMD. These contradictory findings may be due to differences in methods used to measure vitB_{12} such as investigation of dietary intake of vit B_{12}, plasma or serum level of vit B_{12}, analysis of MMA. Also the population which took part in these studies are from different regions that may also be a cause of contradictory findings. The limitation of our study was that due to its limited sample size, the study does not reflect the result of the general population in Bangladesh. We did not measure the Hcy level and MMA to confirm functional deficiency of vit B_{12}. Also the cross-sectional nature of the study does not help to establish the causal relationship between vitB_{12} and BMD. But to our knowledge, this study is the first to be performed to investigate the relationship between BMD and vit B_{12} in postmenopausal women of Bangladesh. As osteoporosis and age-related bone disorders are now a day's becoming a public health burden in the aging Bangladeshi population, we hope our study will help to start additional research for better understanding the role of vitamin B_{12} in bone health in older women in Bangladesh.

CONCLUSION

Vitamin B_{12} is positively associated with bone mineral density and T-score at the lumbar spine and left femoral neck but not at the right femoral neck in postmenopausal women.

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