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The Effect of Hyperhomocysteinemia, Low Serum Vitamin B12 and Folate on Increasing Lose of Bone Density and Increasing Incident of Osteoporotic Hip Fracture among Elderly Population

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Abstract

Subjective: *Many studies shown a relationship between high plasma homocysteine level, low serum vitamin B12 and folate level and increasing risk of loss bone mass density and the increasing risk of incidental hip fracture among the elderly population (age more than 65 years old in both sexes)*

Aim of the study: *This systemic article review aims to revise the main researches related to this subject and find out whether there is a significant relationship between high plasma homocysteine level, low serum vitamin B12 and folate level and increasing risk of loss bone mass density and the increasing risk of incidental hip fracture among the elderly population (age more than 65 years old in both sexes)*

Methods: *About 10retrospective and prospective (cohort) cross sectional related studies have been reviewed and analyzed for the purpose of reaching the results. High plasma homocysteine level (> 12µg/L), low serum vitamin B 12 level (< 200 µg/L)And low serum of vitamin B9(>140 µg/L)*

Discussion: *Multiple medical and nutritional studies talk, discuss, and explain the relationship between high plasma homocysteine level, low serum vitamin B12 and folate level and increasing risk of loss bone mass density and the increasing risk of incidental hip fracture among the elderly population (age more than 65 years old in both sexes)*

Results: *Most of the reviewed related researches have shown mild (weak) relationship between high plasma homocysteine level, low serum vitamin B12 and folate level, but this related researchhas shown mild significant relationship between high plasma homocysteine level, low serum vitamin B12 and folate and the increasing risk of incidental hip fracture.*

Keywords: *Osteoporosis, Vitamin B12, Homocysteine, Folic Acid, Hip Fracture.*

Introduction

Osteoporosis is a skeletal disorder characterized by a loss of bone matrix (osteoid) that reduces bone integrity and bone strength, predisposing to an increased risk of fragility and fracture. In the United States, osteoporosis causes over 1.5 million fractures annually. White women aged 50 years and older (who do not receive estrogen replacement) have a 46% risk of sustaining an osteoporotic fracture during the remainder of their lives. Vertebral fractures are the most common fracture; they are usually diagnosed incidentally on radiographs or CT scanning.[1]

Largely due to a reduction in smoking, the age-adjusted risk for hip fracture has declined in the United States in recent years. However, the risk for fragility fractures. remains high and varies with ethnicity, sex, and age. The lifetime risk of hip fracture is 12.1% in White women and 4.6% in White men. The risks are lower in Hispanic women and men and lower yet in Chinese women and men (with similar gender differences). Blacks also have a lower risk for fracture due to higher BMD and hip morphology that is less fracture prone. There is much less ethnic variability for vertebral fractures. The prevalence of vertebral fractures in women older than 65 years is 70% for White women, 68% for Japanese women, 55% for Mexican women, and 50% in Black women.

Osteoporosis can be caused by a variety of factors (Table 26-10). The most common causes include aging, sex hormone deficiency, alcohol use disorder, cigarette smoking. long-term proton pump inhibitor therapy, and high-dose corticosteroid administration. Women who chronically consume cola beverages are at increased risk for osteoporosis of the hip. Hypogonadal men frequently develop osteoporosis. Anti-androgen therapy for prostate cancer can cause osteo- porosis, and such men should be monitored with bone densitometry.

Aging	**Medications (long-term)**
Alcohol use disorder (alcoholism)	Aromatase inhibitors Corticosteroids
Cigarette smoking Cola consumption in women (hip)	GnRH inhibitors
Ethnicity: White	Heparin
Female sex	Pioglitazone
Genetic disorders	Proton pump inhibitors
Aromatase deficiency	Selective serotonin reuptake
Collagen disorders	inhibitors (elderly)
Ehlers-Danlos syndrome	SGLT2 inhibitors
Homocystinuria	Vitamin A excess, vitamin D
Hypophosphatasia	excess
Idiopathic juvenile and adult	**Underweight (BMI < 18.5) Miscellaneous conditions**
osteoporosis	Anorexia nervosa Celiac disease
Marfan syndrome Osteogenesis imperfecta	Copper deficiency
Hormone deficiency	Cystic fibrosis
Estradiol (women)	Diabetes mellitus
Testosterone (men)	(uncontrolled) HIV infection
Hormone excess	Hyponatremia (chronic)
Cushing syndrome	Inflammatory bowel disease Liver disease
Hyperparathyroidism	(chronic)
Thyrotoxicosis	Mastocytosis (systemic)
Low physical activity and immobilization	Primary biliary cholangitis
Malignancy, especially plasma cell myeloma	Protein-calorie malnutrition
	Rheumatoid arthritis Thalassemia major
	Vitamin C deficiency

Elderly Patients with clinical and angiographic evidence of coronary artery disease and cerebrovascular and peripheral vascular diseases tend to have higher levels of plasma homocysteine than persons without these vascular diseases. Although this effect was initially thought to be due at least in part to heterozygotes for cystathionine beta-synthase deficiency (see below), there is little supporting evidence. Rather, an important factor leading to hyperhomocysteinemia is folate deficiency. Pyridoxine (vitamin B) and vitamin B are also important in the metabolism of methionine, and deficiency of any of these vitamins can lead to accumulation of homocysteine. Several genes influence utilization of these vitamins and can predispose to deficiency. For example, having one copy-and especially two copies-of an allelethat causes thermolability of methylene tetrahydrofolate reductase predisposes people to elevated fasting homocysteine levels. Both nutritional and most genetic deficiencies of these vitamins can be corrected by dietary supplementation of folic acid and, if serum levels are low, vitamins B, and B. 12 In the United States, cereal grains are fortified with folic acid. However, therapy with B vitamins and folate lowers homocysteine levels significantly but does not reduce the risk of either venous thromboembolism or complications of coronary artery disease. The role of lowering homocysteine as primary prevention for cardiovascular disease has. received modest direct support in clinical trials. Hyperhomocysteinemia occurs with end-stage chronic kidney disease. In the general population, elevated homocysteine correlates with cognitive impairment.[(1)]

Many factors contribute to bone health. Bone is a dynamic tissue in a constant state of remodeling. Bone formation exceeds bone resorption generally in the first three decades of life, the age when peak bone mass is achieved. After this time, bone resorption is favored, and bone loss ensues. Osteoporosis is a chronic, multifactorial disorder characterized by low bone mass and microarchitectural deterioration of bone tissue. Deterioration of bone quality predisposes affected individuals to an increased risk of fragility fracture. The most common osteoporotic fracture sites are the spine, hip, and wrist, with both spine and hip fractures accompanied by considerable disability and increased morbidity and mortality, in addition to increased social and economic burden. This burden is expected to increase substantially in Europe in the coming decades due to a rise in life expectancy. Combined supplementation of calcium and Vitamin D have been proven to reduce bone loss and fracture incidence. However, it is possible that nutritional factors not typically linked with bone health could play a protective role for bone. Association studies have identified vitamins related to fractures or bone mineral density. Emerging evidence in groups of healthy individuals suggests a protective association of certain B Vitamins, in particular Vitamin B12 and folic acid, a detrimental effect of homocysteine and the 677C T polymorphism in the gene encoding the folate metabolizing methylene tetrahydrofolate reductase (MTHFR) enzyme. High concentrations of homocysteine and low levels of Vitamin B12 and folate, the main determinants in the metabolism of homocysteine, have been associated with low bone mineral density (BMD) and a higher risk of fractures in the elderly. Analyses of randomized controlled trials have shown that supplementation of folic acid (0.5-5 mg day) has resulted in reducing the levels of homocysteine in blood up to 25%; the co-supplementation of folic acid and Vitamin B12 (0.5-5 mg day and 500 mcg day, respectively) provided a further reduction of 7% with a decrease in serum total homocysteine by 32%. To date, the mechanisms linking homocysteine to increased fracture risk have not yet been clarified. It is known that serum homocysteine is regulated by Vitamin B12 and folic acid, and supplementation with these vitamins decreases serum homocysteine levels. It is also known that folate, Vitamin B12. Vitamin B6 and riboflavin are involved in the metabolism

of an S-containing amino acid, homocysteine. Homocysteine metabolism links the methionine cycle with the folate cycle. A first link between homocysteine (hey) and the skeleton has been noted in studies of hyperhomocysteinuria, a metabolic disorder characterized by exceedingly high levels of plasma and urine. Individuals with hyperhomocysteinuria exhibit numerous skeletal defects, including reduced BMD and osteopenia. Homocysteine comes from the breakdown of methionine, one of the essential amino acids used for protein synthesis. Homocysteine can be converted to cystathionine with Vitamin B6 and further to cysteine. Alternatively, homocysteine can be remethylated to methionine with help from vitamin B12. The latter reaction is catalyzed by methionine synthase and requires 5-methyltetrahydrofolate, the principal circulating form of folate, and Vitamin B12 in its co-factor form, methyl cobalamin. The formation of 5-methyltetrahydrofolate is catalyzed by the MTHFR enzyme.[2]

The pathway for the conversion of homocysteine to methionine is the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine, catalyzed by the Vitamin B12-dependent enzyme, methionine synthase. Reducing elevated homocysteine levels through folic acid and Vitamin B12 supplementation could theoretically ensure proper bone health and prevent osteoporosis. However, at present, no consensus has been reached on the magnitude of the association between Vitamin B12, folate, homocysteine, and bone health, nor on the possible effect of Vitamin B12 and folate supplementation on bone health.

Materials and Methods

Information sources and study design

The PRISMA 2020 guideline was followed to report this systematic review. The search was conducted in the Access, Cambridge Core, Cochrane Library, PubMed, and Science Direct electronic databases between January 2023 and August 2023. We considered randomized controlled trials (RCTs) published in English in the last ten years, screened in filter tools in all databases. A maximum of 8% of the references are from earlier periods, as it is necessary to cite classic literature in this area.

A cross-section retrospective longitudinal follow - up study, carry the Title: plasma B-Vitamins, Homocysteine, and their relationship with Bone Loss and Hip Fracture in Elderly population authored by Robert R. The Objective of this study: to examine the association of plasma concentrations of folate, vitamin B12 and Homocysteine with bone loss and hip fracture risk in elderly population. Participants: A total of 1002 men and women (mean age 75 years) represent part of the community dwellings residents of Framingham, MA, it was included in the study.[3]

Baseline Blood samples were obtained from the population sample for serum vitamin B12 and Homocysteine, Harvard Medical school, Boston, USA.

A cross-sectional (RCTs) longitudinal follow-up case control comparative Framingham study, achieved by (author Robert R. McLean et, al.) (from 1975 till 1979)", which carry the title: Homocysteine as a predictive factor for hip fracture in elderly persons Number of participants 3261 (female: 2043) (males 1218) Age (Mean with SD/Range) 59-91, Both sexes from different races and ethnicity and patients on HRT (estrogen therapy).(4)

Excluded criteria in this study

1. Age <59 years
2. Any patient with history of previous hip fracture
3. patient on recent corticosteroids therapies within the last year.
4. Any patient with history of cancer.

Discussion

This systematic review with dose-response meta-analyses was conducted within the scope of the EURRECA (European Micronutrient Recommendations Aligned) Network of Excellence (http://www.eurreca.org/) [21]. We

followed a standardized methodology which is described in short below.[5]

Search Strategy and Selection of Articles. We conducted systematic literature searches for (1) vitamin B12, (2) folate, and (3) homocysteine. The electronic databases MEDLINE, EMBASE, and Cochrane Library Central were searched, using search terms in "MeSH" terms and "title" and "abstract" on study designs in humans, vitamin B12, folate, homocysteine, and intake or status. The full Medline search strategy is available online, (see Appendix 1 in Supplementary Material at http://dx.doi.org/10.1155/2013/486186).[5]

To be able to use the same search to identify publications on other health related outcomes both in adults and elderly and in younger population groups, no terms were added to limit the search to health outcome or study population. Moreover, by using a broad search we expected a more complete retrieval of relevant publications. In this review only the results on vitamin B12, folate, and homocysteine status (i.e., biomarkers measured in serum or plasma) in relation to bone health indicators (fracture risk and BMD) are presented. In addition to the search, reference lists of 10 review articles were checked to identify potentially relevant references that were not identified with the multidata base search. The search was not limited by language. This review contains studies started from Jan 2023 up to Aug 2023.[5]

We selected articles in two steps. The first selection step included screening for title and abstract by three independent investigators (J. P. van Wyngarden, E. L. Doets, SB). In the second selection step, full texts of the selected abstracts were evaluated on the basis of predefined inclusion criteria by four investigators (J. P. van Wyngarden, E. L. Doets, A. Szczecin ska, MP).[5]

To alignment and quality control 10% of the references in each selection step were screened and selected in duplicate by two investigators independently. Results were compared and discrepancies were resolved by unanimous consensus among all investigators.[5]

Studies were eligible for inclusion if they were conducted in apparently healthy human subjects aged ≥18 y. Furthermore, studies had to report fracture incidence, fracture risk, or bone mineral density (BMD) as a health outcome and had to report baseline data on the outcome measure.[5]

Observational studies were included if theyhad a prospective cohort, nested case-control, or cross-sectional design, and (2) addressed serum/plasma concentration of markers indicating vitamin B12 status (serum/plasma vitamin B12, methylmalonic acid (MMA), holotranscobalamin (holoTC)), folate status (serum/plasma folate or erythrocyte folate), or homocysteine status (serum/plasma homocysteine). Intervention studies were included if they (1) had a randomized controlled trial design, (2) studied the effects of vitamin B12 or folic acid supplements, fortified foods or micronutrient intake from natural food sources and included a placebo or untreated comparison group, and (3) had a minimum intervention duration of six months.[5]

Data Extraction and Statistical Analysis. We extracted data for each of the identified studies on population characteristics, study design, assessment of vitamin B12, folate and homocysteine status, and fracture risk or bone mineral density. [5]

Opportunities for meta-analysis were evaluated based on comparability of health outcome and status marker. If less than three comparable studies were available, results were qualitatively described. If three or more comparable studies were available, the results of these individual studies were expressed in a standardized format to allow comparison in the form of a continuous dose-response meta-analysis that pools the regression coefficient (β) (SE) from multiple adjusted models. We chose to express association measures for serum/plasma vitamin B12 per 50 pmol/L. When βs were not reported in the original article, we transformed Relative Risk (RR), Hazard Ratio (HR), or Odds Ratio (OR) to βs,

using a standardized method. The transformations to obtain βs and SEs and statistical analyses were performed using R statistics version (http://www.R-project.org/), with statistical significance defined as $P< 0.05$. HR and OR were considered as RR because the outcome was relatively rare. If articles reported insufficient data (missing data, inconsistencies, or any other uncertainties), we contacted corresponding authors for additional information.(5)

We calculated summary estimates of comparable studies using random effects meta-analysis. Applying the methods of Der Simonian and Laird, the between study variance was estimated which was used to modify the weights for calculating the summary estimate. Residual heterogeneity between studies was evaluated using Q-statistic and I 2 -statistic see table (1)

Table 1: Studies regarding the association between vitamin B_{12} and bone health.

Author Year	Study characteristics Duration of follow-up (when applicable) Country *Risk of bias*	Population characteristics N (% men) Age (y) ± SD	Vitamin B_{12} status pmol/L* Mean ± SD	Outcome	Association type	Results*
Dhonukshe-Rutten et al. 2005 [31]	Cohort (3 y) The Netherlands *High risk*	1233 (48%) 75.5 ± 6.6	♀: 289 ± 99 ♂: 268 ± 89	Fracture (verified by physician or radiograph)	β (SE) for association $vitB_{12}$-fracture (per 50 pmol/L)	♀: −0.09 (0.06)^{b,1} ♂: 0.02 (0.08)^{b,1}
Gjesdal et al. 2007 [24]	Cohort (12.6 y) Norway *Low risk*	4761 (45%) 65–67 at baseline	♀: 386.4 ± 372.0 ♂: 358.3 ± 276.2	Hip fracture (verified by hospital discharge diagnoses)	β (SE) for association $vitB_{12}$-hip fracture (per 50 pmol/L)	♀: −0.03 (0.03)^{b,2} ♂: −0.06 (0.05)^{b,2}
McLean et al. 2008 [25]	Cohort (16 y) USA *Low risk*	823 (41%) 75.3 ± 4.9	Deficient (<148): ♀9%/♂14.0% Low (148–257.9): ♀24.3%/♂32.5% Normal (≥258): ♀66.7%/♂53.5%	Hip fracture (verified by review medical records)	β (SE) for association $vitB_{12}$-hip fracture (per 50 pmol/L)	♀: −0.09 (0.06)^{c,1} ♂: −0.09 (0.11)^{c,1}
Ravaglia et al. 2005 [26]	Cohort (4 y) Italy *Moderate risk*	702 (47%) 73.0 ± 6.0	Geometric mean (95% CI) 249.1 (203–272)	Fracture (verified by review medical records)	β (SE) for association $vitB_{12}$-fracture (per 50 pmol/L)	0.04 (0.08)^{d,2}
Bozkurt et al. 2009 [32]	Cross-sectional Turkey *High risk*	178 (0%) 53.5 ± 8.0	247.7 ± 85.4	BMD: LS, FN [DXA]	Logistic regression for FN, LS and FN + LS combined for $vitB_{12}$ status under the quintile value. β (SE) + P value	LS: −2.3 (0.9) $P = 0.017$ FN: −0.4 (0.9) $P = 0.669$ LS + FN: 1.8 (0.8) $P = 0.045^{e}$
Bucciarelli et al. 2010 [33]	Cross-sectional Italy *Moderate risk*	446 (0%) 65.1 ± 9.4	(geometric mean ± SD) 399.1 ± 1.6	BMD: FN, LS, TH [DXA, Prodigy, GE, Lunar]	β for association $vitB_{12}$-TH BMD β (SE) (per 50 pmol/L)	−0.00105 (0.939)^{f,1}
Cagnacci et al. 2008 [34]	Cohort (5 y) Italy *Moderate risk*	117 (0%) 54.4 ± 0.5	(Mean ± SE) 548.5 ± 40.5	BMD: LS [DXA: Lunar DPX]	Regression for $vitB_{12}$-BMD change β (SE) P value	−0.003 (0.012) $P = 0.784^{g}$
Dhonukshe-Rutten et al. 2005 [35]	Cross-sectional The Netherlands *Moderate risk*	194 (26%) 78.3 ± 5.5	♀288 ± 131 ♂238 ± 95	BMD: whole body [DXA, Lunar DPX-L]	Multivariate regression, β for association $vitB_{12}$-BMD β (95% CI) in women	♀: $12.3\,10^{-3}$ ($0.2\,10^{-3}$–$2.4\,10^{-3}$)^{h}
Gjesdal et al. 2006 [10]	Cross-sectional Norway *Moderate risk*	5329 (43%) middle aged: 47–50 Older: 71–75	♀393.4 ± 235.8 ♂374.6 ± 230.7	BMD: TH [DXA, Lunar EXPERT-XL]	OR (95% CI) for low BMD per category $vitB_{12}$ status 1: <230 pmol/L 2: 230.0–279.9 pmol/L 3: 280.0–414.9 pmol/L 4: ≥415.0 pmol/L + P for trend	♀: 1: 0.97 (0.68–1.37) 2: 0.87 (0.63–1.21) 3: 1.02 (0.82–1.27) 4: 1.00 (reference) P for trend = 0.61 ♂: 1.22 (0.82–1.81) 1.14 (0.80–1.62) 0.97 (0.74–1.28) 1.00 (reference) P for trend = 0.25^{i}

Results

We identified 180 studies after removing duplicate trials (n = 137). The articles that constituted the results of this review are at the end of the flowchart. A total of 10 RCTs met the inclusion criteria, The study found that plasma homocysteine concentrations are associated with the risk of hip fracture in both genders. The study participants with higher homocysteine concentration have a significant relationship with hip fracture.

Comparing with the population sample participants who have the lowest plasma homocysteine level: by a risk factor of almost four in men and by a risk factor of 1.9 in women. The risk of hip fracture was elevated by 59 percent in men and by 26 percent in women for each increase of 1 SD in the log-transformed total homocysteine concentration.

A cross-sectional Randomized double - blind placebo-control study which carry the title: Effect of daily of vitamins B12 and folic acid supplementations on fracture incidence in elderly individuals with an elevated plasma homocysteine concentration, authorized by (Jameke P van Wyngarden, et al.), over a period of 2 years, in Netherlands, Authorized by (Jameke P van Wyngarden, et al.), over a period of 2 years, in Netherlands. Design of this study: This double- bind randomized, placebo-controlled trial in 2919 aged>=65 years with elevated plasmahomocysteine concentrations (12-

50µmol/L). Participants were assigned to receive daily 500 micgm vitamin B12 + 400 micgm folic acid or placebo supplementations for 2 years. Both intervention and placebo tablets also contained 600 IU Vitamin D 3.

The primary endpoint was the time to first osteoporotic fracture. Exploratory perceived subgroup analyses were performed in men and women and in individuals. The result of this study was: Osteoporotic fracture occurred in 61 persons (from total 2919 participants) representing (4.2%) in intervention group and 75 persons represent (5.1%) in the placebo group. Osteoporotic fracture risk Was not significantly different between groups in the intention-to-treat analysis. Total number of participants for both genders 2919.

Fractures

Vitamin B12. Four longitudinal observational studies [3, 24–26], including 7475 elderly people with 3 to 16 years of follow-up and a total of 458 cases addressed the association between serum/plasma vitamin B12 and fracture (Table 1). Pooled analysis of the association between 50 pmol/L increase in plasma/serum B12 and change in fracture risk showed an inverse association (RR = 0.96, 95% CI = 0.92 to 1.00) with no heterogeneity between studies ($I2$ = 0%, P = 0.76) (Figure 2). This indicates that a vitamin B12 increase of 50 pmol/L tends to decrease the risk of fracture with 4%.

Folate. Most of the related across sectional longitudinal studies shows mild significant association was observed between low serum folic acid and increase the risk of incidental osteoporotic hip fracture.

Homocysteine. Eleven longitudinal observational studies examined the association between homocysteine status and fracture incidence. A meta-analysis of eight studies, including 11511 elderly people with 3 to 12.6 years of follow-up and 1353 cases, showed mildly significant fracture risk with increasing plasma homocysteine.

Conclusion

Most of the reviewed related research have shown mild (weak) relationship between high plasma homocysteine level, low serum vitamin B12 and folate level, but this related research has shown mild significant relationship between high plasma homocysteine level, low serum vitamin B12 and folate and the increasing risk of incidental hip fracture.

The recommendation is to put consideration by checking serum vitamin B12, folic acid, vitamin B6(pyridoxine) or requesting for plasma homocysteine level for either border line serum vitamin B12, folic acid and/or vitamin B6 among all geriatric patients attending to your clinic for any reason as part of preosteoprotic and its related fracture risk screening task and managing their abnormal results effectively.

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