

HYPERHOMOCYSTEINEMIA AND BONE MINERAL DENSITY: A CASE - CONTROL STUDY

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ABSTRACT

Objectives: To find out whether homocysteine has a direct effect on bone or it is an innocent bystander? The study was designed to investigate probable role of homocysteine on bone mineral density (BMD).

Methodology: This a case-control study wherein, 30 patients with at least one densitometry criterion of osteoporosis in femoral neck or lumbar spine were enrolled as the case group along with another 30 normal subjects with normal BMD, as the control group. The patients of the two groups were matched for their ages and sex. In all eligible patients BMD was measured by DEXA and fasting serum homocysteine level were measured by Enzyme Immunoassay Kit.

Results: The mean of serum level of homocysteine were 11.67 ± 4.38 and 11.97 ± 3.09 $\mu\text{mol/l}$ in control and case groups respectively. The difference between two groups was not significant ($P=0.761$). Serum homocysteine level and BMC of various areas in case and control groups had no significant correlation [lumbar spine in control group ($r=0.025$, $p=0.9$), lumbar spine in case group ($r=0.071$, $p=0.716$), femoral neck in control group ($r=0.276$, $p=0.147$), femoral neck in case group ($r=0.001$, $p=0.998$)].

Conclusion: Despite numerous studies about direct effect of homocysteine on increase of osteoporotic fracture risk, our study did not show a correlation between serum level of homocysteine and BMD. Due to multiplicity of factors affecting bone density, final conclusions need extensive investigations with attention to other confounding factors.

KEYWORDS: Homocysteine, Osteoporosis, Bone Mineral Density.

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INTRODUCTION

Osteoporosis is the most common metabolic bone disease, and involves one third of women and one eighth of men.¹ One of the best and

profitable measures of the approach to this enlarging epidemic health problem is prevention through detection and elimination of correctable pathogenic factors. Homocysteine is one of this factors that has recently attracted scientists attention. Although a lot of investigations have been accomplished in this filed, due to multiplicity of factors that play a role on bone metabolism, including homocysteine, more studies seems to be necessary for precise conclusions.²

In spite of the fact that homocysteine do not exist in human diet, it is an essential interme-

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diate in methionine metabolism in mammals.³ Increased frequency of osteoporosis was discovered for the first time in patients with homocystinuria (in them serum level of homocysteine is high).⁴ Increased serum level of homocysteine causes derangement in collagen cross linking, which results in weakness of bone matrix.⁵⁻⁷ These results have obtained from patients with homocystinuria or with methylene tetrahydrofolate reductase (MTHFR) gene polymorphism (defective homocysteine metabolism). Some studies have shown that in normal population the serum level of homocysteine do not reach to such a high level as homocystinuria, and can not affects bone structure.^{4,8}

Many factors contribute to plasma homocysteine level⁹ such as

- 1) Congenital abnormalities in homocysteine metabolism
- 2) Enzyme polymorphisms due to defects of MTHFR gene
- 3) Alcohol abuse
- 4) Folate deficiency
- 5) Vitamin B₆ or B₁₂ deficiency
- 6) Hypothyroidism
- 7) Drugs such as isoniazid
- 8) Background diseases such as leukemia, renal failure, renal transplantation
- 9) Sampling or assay errors.

Attention to relationship between homocysteine and osteoporosis does not have a longstanding history. Studies in this filed have not obtained a precise and applied results. Especially we have not sufficient data about this problem in our region. Have homocysteine a direct effect on bone or it is an innocent bystander? This is the question we tried to answer in this study.¹⁰ The present study has been designed to investigate probable role of homocysteine on bone mineral density (BMD).

METHODOLOGY

It is a case control study conducted during one year from June 2005 to May 2006 among patients presenting to Densitometry Center of Endocrine Section of Sina Teaching Hospital in Tabriz-Iran. Sixty patients were selected and

assigned in two case and control groups. The case group included 30 subjects with osteoporosis based on WHO definition of BMD categories¹¹ at least in one region in femoral neck or lumbar spine areas. Control group included 30 subjects with normal BMD. Subjects were age and sex matched in two groups. All subjects were questioned about their level of physical activity (categorized as "usual house-keeping" and "higher level physical activities") and daily sun exposure (categorized as "less than 15 minute per day" and "greater than 15 minute per day"). Also a brief dietary history was taken about usual consumption of food and beverages such as caffeine, tea, spirits, and daily use of milk or milk products. Any person with unusual dietary habits were excluded. Cigarette smoking status was also disclosed.

BMD had been determined in lumbar spine and femoral neck areas using Dual Energy X-ray Absorptiometry (DEXA) by LUNAR version DPC-MD apparatus. For measurement of serum homocysteine level sampling had been performed after 10 to 12 hour over night fasting. Serum homocysteine level had been assayed by a standard Enzyme Immunoassay Kit (DRG Diagnostics, USA). Hyperhomocysteinemia was defined as serum homocysteine level higher than 14 μ mol/L in males and 12 μ mol/L in females.¹² The main inclusion criteria were primary osteoporosis in case group. Patients with secondary osteoporosis were excluded (subjects with past medical history of any medical conditions affecting bone density such as rheumatologic, endocrinologic, hematologic, renal, gastrointestinal, and liver disorders were excluded). Subjects with history of consumption of medications effective in BMD or plasma homocysteine level including corticosteroids, heparin, thyroxin, anticonvulsants, aluminum containing antacids (continuous use for longer than one year duration), long acting GnRH analogues, estrogen, multivitamins, folic acid, and vitamins B₆ or B₁₂ were excluded in both groups. The study protocol was approved by regional ethics committee, and a written consent was taken from all patients to participate in the study.

Table-I: Comprison of studied variables in case (osteoporotic) and control (normal BMD)

Variable	Control Group	Case Group	P-value
Age	59.96±9.75	62.90±11.63	0.295
Age of Menarche	13.86±1.01	14.62±1.14	0.017
Age of Menopause	47.41±4.77	48.76±5.47	0.467
Weight(kg)	72.93±11.94	63.31±9.87	0.001
Height(cm)	156.0±8.19	152.24±4.66	0.035
Body Mass Index(kg/m ²)			
Sex			
Male	6	1	
Female	24	29	0.103
Daily Milk Consumption	7(14.1%)*	8(26.7%)	
	7(14.1%)**	12(40%)	0.306
	15(51.8%***)	10(33.3%)	
Physical Activity	11(36.7%)+	26(86.07%)	0
	19(63.3%)++	4(3.13%)	
	6(20%)^	15(50%)	0.015
Daily Sun Exposure	24(80%)^^	15(50%)	
Serum Homocysteine Level(μmol/l)	11.67±9.75	11.97±3.09	0.761
Hyperlipidemia†	8(26.7%)	14(46.7%)	0.108

P<0.05 Considered significant, *Less than three glass per week, **3 to 7 glass per week, *** Greater than 7 glass per week, +Usual housekeeping, ++Higher level physical activities, ^Less than 15 minute per day, ^^Greater than 15 minute per day, †LDL >130mg/ dl, Triglyceride>150, HDL<40 for males <50 for females.

Statistical analysis was preformed by SPSS version 14 software. Quantitative data presented as mean ± standard deviation (SD) and qualitative results as frequency. Chi square, Fisher's exact, Independent sample tests; Pearson's correlation, and Multivariate Logistic Regression Analysis have been used as needed. Results were considered significant when P Value was less than <0.05.

RESULTS

Mean age of study population was 61.43±75 years. Mean age of menarche was 14.28±1.24 years. Mean age of menopause was 48.34±5.24 years. The rate of hyperhomocysteinemia was 9(53.3%) and 14(70%) in control and case groups respectively. There were not significant difference between them (P=0.184). The mean of Bone Mineral Content (BMC) of lumbar spine in control group was 39.35±10.55 g and 22.14± 6.04g in case group (p <0.001). The respective rates for femoral neck were 40.01±9.46 g and 23.21±5.14 g (p<0.001). The mean of serum level of homocysteine were 11.67±4.38 and

11.97±3.09 μmol/L in control and case groups respectively. The difference between two groups was not significant (P=0.761). We did not find significant correlation between serum homocysteine level and subjects age(r= -0.035, p=0.793). Correlation between serum homocysteine level and BMC of various areas in case and control groups was as follows:

- 1) BMC of lumbar spine in control group (r= 0.025, p=0.9) (Fig-1).
- 2) BMC of lumbar spine in case group (r=0.071, p=0.716) (Fig-2).
- 3) BMC of femoral neck in control group (r=0.276, p=0.147) (Fig-3).
- 4) BMC of femoral neck in case group (r=0.001, p=0.998) (Fig-4).

Comparison of studied parameters is summarized in Table-I (univariant analysis). For elimination of effects of different variable on each other, parameters that have shown significant difference between two groups were entered in a multivariate analysis (for detection of an independent effective factors). The results showed that there was no significant

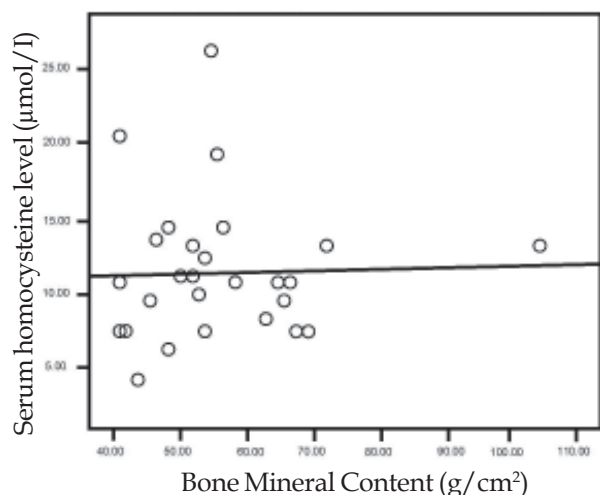


Figure-1: Correlation between serum homocysteine level and BMC of lumbar spine in control group ($r=0.025$, $p=0.9$)

differences between two groups in daily sun exposure, mean of BMI (Body Mass Index), mean of weight, and height ($p>0.05$). The mean age of menarche was significantly higher in case group ($p=0.010$). While subjects of control group had greater physical activity ($p=0.010$).

DISCUSSION

Currently, the role of hyperhomocysteinemia is generally accepted as a risk factor for cardiovascular and thromboembolic disorders. There are other studies that show the effects

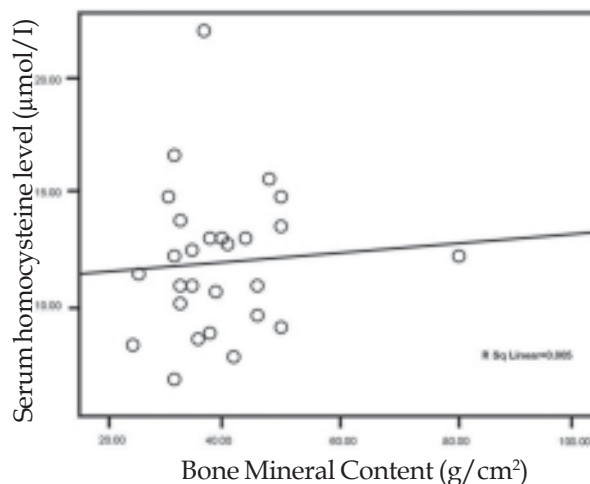


Figure-2: Correlation between serum homocysteine level and BMC of lumbar spine in case group ($r=0.071$, $p=0.716$)

of this novel factor in other diseases such as neurological disorders, pernicious anemia, renal failure, hypothyroidism, malignancies, and some dermatologic - rheumatologic disorders.¹³⁻¹⁶ One of the subjects that has recently come under intense discussion is the role of homocysteine in osteoporotic fractures. Although a lot of studies have been conducted in this field but due to wide range of interactions of this compound with some other organic components of bone, a precise and applicable results have not been obtained.^{10,17-19}

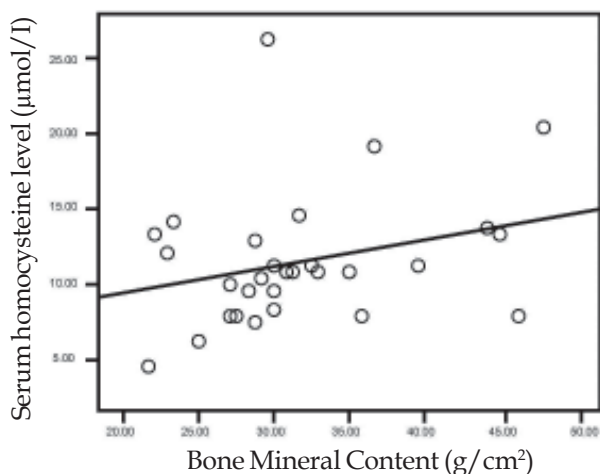


Figure-3: Correlation between serum homocysteine level and BMC of femoral neck in control group ($r=0.276$, $p=0.147$)

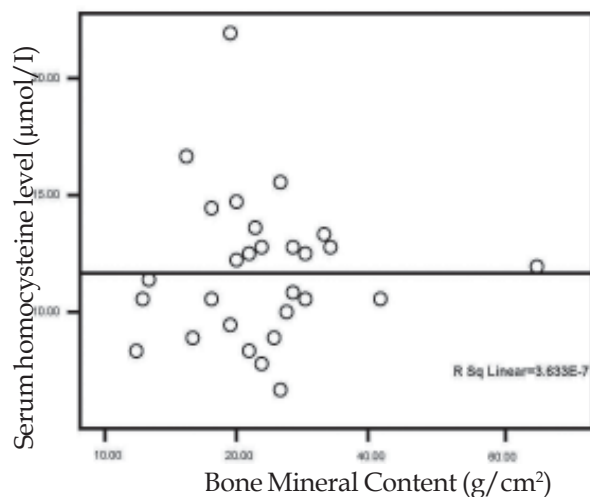


Figure-4: Correlation between serum homocysteine level and BMC of femoral neck in case group ($r=0.001$, $p=0.998$)

In separate investigations Janson, McLean and van Meurs have shown that increased level of serum homocysteine can increase risk of osteoporotic fractures^{4,16,20} Also Sato in a study on 433 Japanese patients with history of stroke concluded that hyperhomocysteinemia is one of the risk factors for hip fractures in stroke patients.

Dhonukshe-Rutten studied 1267 patients in the Netherlands and found that presence of hyperhomocysteinemia has increased risk of fracture rate but there were not correlation with BMD.²¹ In studies conducted by Browner, Cagnacci, Herrman and Viladsen correlation between serum levels of homocysteine and BMD were not seen.^{19,22-25}

It seems that there is a correlation between increased level of homocysteine and fracture rate, but such a correlation between homocysteine and BMD do not exist. Some reasons have been mentioned to explain this relationship. In some studies attention has been paid to folic acid and other group B vitamins. It has been shown that something that cause fracture in some patients were not homocysteine itself but other nutrient deficiencies such as folic acid and group B vitamins which were the main cause. Serum level of homocysteine could be an index of such nutrients deficiencies^{4,23,26} In a study by Golbahar et al on 271 Iranian post-menopausal women bone mineral densities at both neck of femur and lumbar spine were significantly and negatively correlated with the logarithm of plasma total homocysteine. Bone mineral density at the lumbar spine was also significantly and positively associated with plasma folate. However, no correlation between MTHFR polymorphism with bone mineral density at neck of femur and lumbar spine was observed. The negative association of plasma total homocysteine with bone mineral density was no longer significant when adjusted for folate and vitamin B₁₂.²⁷

In conclusion as other studies, our study also can not show a correlation between serum level of homocysteine and BMD. As mentioned earlier multiple factors affects bone density. These effects influence bone through complex path-

ways. Thus final conclusion in this field needs extensive investigations with attention to other multiple probable factors. The main drawback of our study was the small sample size. We recommend similar investigations with larger sample size and longitudinal studies for clarification of role of serum level of homocysteine on fracture risk.

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