The Association between Hypercholesterolemia and Reduced Bone Density in the Femoral Head and Lumbar Spine Using Dual-Energy X-Ray Absorptiometry in Postmenopausal Women

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ABSTRACT

Background & Objective: Some studies have suggested the association between the risk of osteoporosis and atherosclerosis. So, we aimed to compare the serum lipid levels of postmenopausal women with reduced bone density to serum lipid levels of those with normal bone density.

Materials & Methods: In this cross-sectional study, all 48-65 year-old women, menopause for more than 1 year prior to the study, who referred to Akbarabadi Hospital, Tehran, Iran, during September 2011-March 2013 were recruited. They underwent bone densitometry using the Dual-Energy X-Ray Absorptiometry Method and were divided into two groups: normal density (control group) and low density (case group). Body mass index (BMI) and waist circumference were measured. After 14 hours, fasting serum levels of lipid, fasting blood sugar (FBS), and HbA$_1$C were checked. Hypercholesterolemia, as low high density lipoprotein (HDL) (<35 mg/dL) and high cholesterol levels (>200 mg/dL), were compared between the groups in addition to low density lipoprotein (LDL) levels.

Results: The data of 241 women were analyzed. The mean±SD levels of serum TC were 192±24.7 and 185±19 mg/dL, in the case and control groups, respectively (P=0.009), and that of serum LDL levels were 112±20.2 and 105±17 g/dL, respectively (P=0.005). There was a significant and negative correlation between the women's Z-score and their cholesterol level (r=-0.162, P=0.012). Regression results revealed that the following factors significantly affected Z-score: BMI, LDL, TC, and duration of menopause.

Conclusion: LDL and TC levels were higher in menopausal women with reduced bone density, which indicates the relationship between hypercholesterolemia and reduced bone density.

Keywords: Bone density, Hypercholesterolemia, Menopause, Osteoporosis

Introduction

Osteoporosis is the most common chronic metabolic bone disease, affecting one in three women over the age of 50, which can remain asymptomatic until the occurrence of fracture **(1)**. It can cause enormous health and economic burden to the patient and the society, as it increases the need for long-term care as well as patients' mortality and imposes great economic burden, estimated to reach $25.3 billion (annual direct costs) by 2025 **(2)**. Treatment of osteoporosis includes pharmacological therapy, which aims to reduce the risk of fracture **(3)**. However, the efficacy of treatment should be examined by incidence of fractures and bone markers, like mineral density (BMD) measurement **(4)**. Accordingly, research has focused on the risk factors to take a step towards prevention. In addition, the risk factors determine who should be evaluated for diagnosis of osteoporosis **(5)**.

Several risk factors have been determined for altered bone metabolism, which predispose the patients to osteoporosis, including genetics, age, sex, race, reproductive status, low calcium intake, and exercise **(6)**. In addition to these factors, there are several diseases, such as obesity and diabetes mellitus, which can affect bone health and thus, increase the risk of osteoporosis **(7)**. Hypercholesterolemia, defined as high levels of total cholesterol (TC) and low density lipoprotein (LDL), is one of the important diseases, frequently observed in the elderly, especially post-menopausal women **(8)**, who are at a high risk of osteoporosis, as well. It has been previously shown that

the risk of cardiovascular diseases is higher in patients with osteoporosis (9). Furthermore, dyslipidemia has also been related to impaired bone metabolism and osteoporosis, has been hypothesized to underlie the shared immunological and inflammatory factors (10). The results of animal studies have indicated that high cholesterol diet, resulting in the production of lipid oxidation products, can impair bone regeneration, reduce bone mineralization, and affect bone mechanical strength (11, 12). Moreover, administration of cholesterol reducing agents such as statins, resulted in significant increase in BMD and reduced the risk of bone fractures (13-15).

Clinical human studies have mainly addressed postmenopausal women, but have reported controversial results about the association between serum lipid profile and BMD or risk of osteoporosis. Some researchers report the adverse effect of hypercholesterolemia (TC and LDL) on BMD (16), while others report no association between serum levels of lipids and BMD (17). Moreover, some also report positive association of higher levels of serum triglyceride and cholesterol with BMD (18). Considering the controversies about the relationship between serum lipids and BMD, and the significance of studying risk factors of osteoporosis for its prevention, as well as the controversies in the results of previous studies, it is necessary to determine the association between serum lipid profile and BMD of postmenopausal women (19). Therefore, in this study, we aimed to compare serum lipid levels of postmenopausal women with reduced bone density with that of participants with normal bone density to investigate the association between serum lipid profiles and BMD.

Materials and Methods

In this cross-sectional study, all menopausal women who referred to Akbarabadi Hospital, Tehran, Iran, from September 2011 to March 2013 were considered as the study population and enrolled into the study based on the following inclusion criteria using convenient sampling method: women aged 48–65 years, menopause for more than one year prior to the study, who referred to the study place during the study period and gave written consent to participate in the study. The study protocol was approved by Iran University of Medical Sciences (ID: 949).

Women with any of the following conditions were not enrolled into the study: participants who experienced menopause before the age of 40, participants with a positive history of cancer, thyroid disease, chronic liver and kidney diseases, bilateral salpingo–oophorectomy before menopause, lumbar spine, hip or femoral head surgery, cardiac artery bypass grafting, history of using bisphosphates or other drugs affecting bone and lipid metabolism, use of anti-diabetic, anti-hypertensive, anti-osteoporosis, or lipid-lowering medications, or hormone replacement therapy.

Considering the correlation coefficient between LDL and bone density and with respect to the correlation coefficient table, the sample size was calculated to be 153 individuals. Participants' information was collected using a questionnaire, designed by the research group, to collect participants' demographics, medical history of underlying diseases (diabetes mellitus, hypertension), parity, and age of menopause. Then the participants underwent bone density evaluation using the Dual Energy X–Ray Absorptiometry (DXA) method and were divided into two groups according to their BMD: participants with normal density (Z-score of -1 to 0) were considered as the control group and participants with low BMD (Z-score of -2.5 to -1 was considered as osteopenic and Z-score<-2.5 was considered as having osteoporosis) as the case group. The two groups were matched in terms of age.

After 14 hours of fasting, a venous blood sample was taken from the participants in the sitting position, collected in normal laboratory tubes, centrifuged for separation of serum, and sent to the laboratory for measurement of serum lipid profile by BT3500 (Biotecnica Co., made in Italy), including (TC, LDL, and high density lipoprotein (HDL)), fasting blood sugar (FBS), and hemoglobin A_1C (HbA_1C), using photometry method. According to the laboratory's kit, the normal range of LDL was 40-150 mg/dL. That of FBS was 70-100 mg/dL, and that of HbA_1C was 3-6%. Hypercholesterolemia was defined as low HDL (<35 mg/dL) and high cholesterol (>200 mg/dL). HbA_1C levels ≥7%, FBS levels ≥126 mg/dL, LDL levels ≥100 mg/dL, HDL levels ≤45 mg/dL, and triglycerides (TG) levels ≥150 mg/dL were considered as abnormal. The researcher also measured the participants' height and weight, to calculate their body mass index (BMI) and waist circumference (WC) and recorded them in the study checklist. BMI values of 18.99 to 24.99 kg/m^2 were considered as normal weight, 25-29.99 kg/m^2 as overweight, and >30 kg/m^2 as obese.

Statistical Analysis

Data were analyzed using PASW software, version 19. Mean and standard deviations (SD) were used for descriptive analysis of quantitative variables and frequencies (percentage) for qualitative variables. Moreover, t test and Chi-square test were used for comparison of quantitative and qualitative variables between the groups, respectively. Pearson's correlation coefficient and logistic regression were used for studying the association of variables with BMD. In all tests, P-value<0.05 was considered as statistically significant.

Results

This study consisted of 241 women including 86 women in the case group and 155 in the control group. The mean and SD values of the participants' demographics are shown in Table 1. As indicated, mean age of the participants (P=0.384) and mean number of parity (P=0.173) were not different between

the groups. The women's mean BMI of was significantly higher in the control group (*P*<0.001), and mean duration of menopause was higher in the case group (*P*=0.007, Table 1).

The mean±SD of HbA₁C levels (*P*=0.807) and FBS levels (*P*=0.601) were not different between the groups. However, the mean±SD of TC levels in the case and control groups were 192±24.7 and 185±19 mg/dL, respectively (*P*=0.009), and that of serum LDL levels were 112±20.2 and 105±17 g/dL, respectively (*P*=0.005). Meanwhile, TG (*P*=0.180) and HDL levels (*P*=0.498) were not different between the two groups.

The results of Pearson's correlation coefficient showed a significant and negative correlation between the women's Z-score and their cholesterol level (*r*=-0.162, *P*=0.012). The results of logistic regression revealed the following factors affecting Z-score: BMI, LDL, parity, and cholesterol (Table 2). There was a negative correlation between cholesterol and Z-score, controlling the effect of age (*r*=-.154, *P*=0.017), BMI (*r*=-.192, *P*=0.003), and duration of menopause (*r*=-.152, *P*=0.019). Multivariate analysis showed that only cholesterol and BMI were associated with Z-score (*P*=0.029 and 0.001, respectively, Table 3).

Table 1. Comparing the mean values of different variables between the two study groups

Variable	Case group (N=86)	Control group (N=155)	P-value*
Age	56.18±5.22	55.57±5.2	0.384
Parity, number	5.00±2.54	4.56±2.23	0.173
Duration of menopause	5.36±7.63	4.79±5.81	0.007
BMI, kg/m²	26.30±3.99	30.22±4.26	0.001
FBS, mg/dL	91.12±10.7	89.99±18.40	0.601
HgA₁C, mmol/mol	5.61±0.77	5.59±0.65	0.807
HDL, mg/dL	46.41±1.40	46.18±1.10	0.149
LDL, mg/dL	112.84±20.27	105.88±17.20	0.008
Cholesterol, mg/dL	192.96±24.72	185.45±19.21	0.009
TG, mg/dL	168.54±35.67	166.71±39.91	0.724
Z-Score	-1.47±0.44	0.133±0.68	0.601

*The results of *t* test, P-values are considered as significant when <0.05; all values are reported as mean ± Standard Deviation
Abbreviations: BMI: body mass index; FBS: Fasting blood sugar; HbA₁C: hemoglobin A₁C; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides

Table 2. The results of regression analysis for the correlation of Z-score with different variables

Z-score	Age	Parity	Duration of menopause	Body mass index	Hemoglobin A1C	High density lipoprotein	Low density lipoprotein	Cholesterol	Triglyceride	Fasting blood sugar
Pearson's correlation coefficient	-.052	-.136	-.106	.413	.006	-.106	-.147	-.162	-.039	-.088
P-value	0.425	0.034	0.100	<0.001	0.931	0.101	0.203	0.012	0.549	0.175

Table 3. The results of multivariate regression for the association of variables with Z-score

	Unstandardized coefficients		Standardized coefficients	*t*	P-value	95% confidence interval	
	B	Std. error	Beta			Lower bound	Upper bound
Constant	-1.591	.837		-1.901	0.059	-3.240	.058
Cholesterol	-.009	.004	-.207	-2.198	0.029	-.018	.000
Low density lipoprotein	.002	.005	.042	.451	0.652	-.008	.012
Body mass index	.088	.013	.409	6.874	0.000	.063	.114

	Unstandardized coefficients		Standardized coefficients	t	P-value	95% confidence interval	
	B	Std. error	Beta			Lower bound	Upper bound
Duration of menopause	-.007	.014	-.035	-.502	0.616	-.033	.020
Age	.006	.013	.032	.454	0.650	-.020	.032
Parity	-.031	.026	-.075	-1.223	0.223	-.082	.019

Discussion

We aimed to assess the association between serum lipid profiles and bone density in menopausal women. For this purpose, we compared the results of serum lipid profiles between the case group (with reduced BMD) and the control group (with normal BMD), categorized based on the results of DXA test. The results indicated that the groups were similar in terms of mean age, parity, HbA₁C, and FBS, while mean BMI and duration of menopause were different between the groups. Accordingly, we adjusted the association based on factors that differed between the groups to investigate the pure effect of serum lipid profiles on BMD. As indicated in the results, the serum levels of TC and LDL were significantly lower in participants with reduced BMD, while TG and HDL levels were not different between the groups. Further analysis showed that LDL and TC had a negative association with Z-score, which remained significant after adjusting for the confounding effect of age, BMI, and duration of menopause. These results showed the adverse effect of TC and LDL on BMD.

Various studies have tested the effect of serum lipid profile on BMD levels and the relationship between hypercholesterolemia and osteoporosis/osteopenia. However, different studies have found different results. Atherosclerosis (carotid intima media thickness) has shown to be negatively associated with BMD in postmenopausal Moroccan women (20), which is similar to the general results of our study, although lipid profiles have not been directly measured in their study. Other researchers have also documented that TC increase the risk of low BMD, suggesting that atherogenic lipid profile induces osteoporosis by oxidative products and inflammation (21), which confirms the results of our study, although the methods used in these studies are different from that of ours (20, 21). Another study in Japan showed that hypercholesterolemia, independent of cytokines, reversely affected bone density and accelerated bone loss in postmenopausal women (22). Similar to the methods used in our study, Ersoy and colleagues investigated 142 postmenopausal women, divided into with and without osteoporosis based in their BMD value and documented no difference in TG levels of the groups, while TC and LDL were different between the groups. Nevertheless, they reported lower TC and LDL levels in women with osteoporosis (23), while in the present study, TC and LDL levels were higher in this group. This difference could be because of the cut off level used for categorizing participants into women with osteoporosis (case group) and normal women (control group); as they

have considered T-score≥1 SD as normal women and T-score≤2.5 SD as women with osteoporosis, while we have considered Z-score for categorization. Similar to the results of the study by Ersoy and colleagues, other researchers have also documented a positive association of BMD with serum TG and TC, which suggests the protective effect of lipids on osteoporosis (18); nevertheless, in our study, we found a negative association between lipids and BMD, which suggests the adverse effects of hypercholesterolemia on osteoporosis. In the National Health and Nutritional Examination Survey (NHANES), 13,592 participants showed negative association of LDL levels with BMD and positive association of HDL with BMD, which lost their significance in fully adjusted model (24). Although the unadjusted results of their study are similar to ours, the adjusted results are dissimilar to ours, as in our study BMD had a negative association with LDL and TC, even after adjustment for age, BMI, and duration of menopause. This difference could be because of the different factors used for adjustment. In a study by Ghadiri–Anari *et al.*, investigation of 170 women aged 50-70 years showed that none of the serum lipids (TG, TC, HDL, and LDL) were associated with participants' femoral and lumbar BMD after adjustment for weight and BMI, while they reported an unadjusted negative association between TC and femoral BMD (17). These results are inconsistent with that of ours, which could be because of the fact that in their report, they assessed the mean serum levels of lipids with mean Z-score, while we have divided the participants into two groups based on their Z-score and compared the serum parameters between them. Sabouri *et al.* have also evaluated 85 male participants with spinal cord injury and have documented no association between TC or LDL with BMD, while HDL was positively correlated with femoral neck BMD (25). The different results obtained in their study compared with ours could be because of the different study population, as they investigated male patients with spinal cord injury, while we included healthy postmenopausal women. This discrepancy in the results of studies could be because of the differences in the characteristics of the study populations, as osteoporosis is a multifactorial disease that can vary based on the risk factors. In our study, the groups were matched in terms of demographic characteristics, such as age and parity, and we used appropriate inclusion criteria to reduce the effect of variables affecting BMD, such as history of hormone replacement therapy, in order to reduce the confounding effect of variables on the study outcome.

In the present study, we excluded any disease or factor identified as a risk factor leading to reduced bone density. Age is an important risk factor for osteoporosis and age-standardized scores are used for assessing BMD (26). Furthermore, the groups in our study were matched in terms of age, and we adjusted the effect of age on the study outcome in the final regression model. Parity has also been identified as an important factor for osteoporosis (27). In our study, the groups had similar mean number of parity. Duration of menopause is also identified as an important risk factor for osteoporosis, which is because of the effect of estrogen deficiency on bone health (28). In our study, duration of menopause was higher in the case group; therefore, the final regression model was adjusted for this factor to indicate the pure effect of lipids on BMD. Moreover, obesity is suggested as another important factor affecting BMD, supposed to underlie the increased inflammation in obese patients (29). In our study, mean BMI of the control group was significantly higher than the case group, while serum levels of lipids were higher in the case group. Thus, we adjusted the regression for BMI, as well. Other factors, such as diabetes have also significant effect on BMD (30). In our study, mean FBS and HbA$_1$C levels were not different in women of the two groups. Therefore, diabetes did not have any confounding effect on the results of our study, and adjusting the results of regression in our study based on age, BMI, and duration of menopause showed that TC and LDL (as the atherogenic cholesterol) levels, important in the occurrence of atherosclerosis, had a negative association with BMD.

The strength of our study was studying two groups with normal and decreased BMD, while the groups were matched in terms of age, parity, FBS, and HbA$_1$C levels. However, this study could have some limitations. The first limitation of this study refers to the non-random inclusion of the participants from one center into the study, which limits the generalizability of the results. Also we could not allocate the participants into the case and control groups by random sampling method, as we categorized them based on the results of BMD. Considering the fact that osteoporosis is a multifactorial disease, there could be other factors affecting the study outcome, although we considered several criteria for inclusion of the participants into the study and controlled the results for other factors. The limited sample size of our study was also one of the limitations.

Conclusion

In conclusion, the higher serum levels of LDL and TC in postmenopausal women with reduced BMD compared with those with normal BMD, and the negative association of LDL and TC with BMD indicate the adverse effect of hypercholesterolemia on BMD, while other factors such as BMI and duration of menopause were also effective in reducing bone density. One of the reasons for the different results in previous studies could be the multifactorial nature of osteoporosis and different characteristics of the study participants. Thus, in the present study, we adjusted the results of regression based on the significant factors to identify the pure effect of lipid profiles on BMD and the effect of TC and LDL on BMD remained significant, even after adjustment. The results of our study indicated the adverse effect of hypercholesterolemia on BMD. Meanwhile, more extensive research is required in this area to study the molecular basis of this association and suggest lipid-lowering drugs as an effective agents for prevention of osteoporosis.

Acknowledgments

Conflict of Interest

The authors declared no conflict of interest.

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