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Original Article

The Relation of the Plasma Ascorbic Acid to Bone Mineral Density in Women Referring to Jami Clinic 2006 Tehran, Iran

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Abstract

Osteoporosis is a silent epidemy of today's world, and is also a prevalent problem of health in Iran. The role of nutrients such as ascorbic acid, a key antioxidant vitamin, in this disease has recently attracted researchers' attention. In this study, we studied the relation between plasma ascorbic acid concentration and bone mineral density (BMD) in women referring to Jami clinic, Tehran, Iran. The study was performed in 200 participants who underwent a fasting blood withdrawal. The control group (n=76; 39.6%) were normal in both lumbar spine (L_1-L_4) and femoral neck (T-score $\geq$ -1). Femur T-score was considered as criterion in selection of the patient group. Seventy six subjects (39.6%) with T-score>-1 were known as total patient group (TP). TP were divided into mild patients (M; -1>T-score -1.7), and severe patients (S; -1.7>T-score). Plasma ascorbic acid levels were analyzed by ferric reducing ascorbic acid concentration, a spectrophotometric assay. Mean SD plasma level of ascorbic acid was 74.55±67.60 µM in control group, 54.73±46.65 M in TP (T-score<-1), 47.31±36.07 M in S patient group (T-score -1.7), and 70.28±61.58 M in M patients (-1>T-score > -1.7), resectively. Our results support an association between plasma levels of ascorbic acid and BMD. The results showed that: 1) plasma levels of ascorbic acid were lower in patients with osteoporosis than in control; 2) the difference was higher between control and group S (T-score $\leq$-1.7) who had more acute disease than group M (-1.7<T-score <-1) who had milder disease; and finally 3) femoral neck T-score showed a positive and significant correlation with plasma levels of ascorbic acid in all participants.

Keywords: Ascorbic acid; Bone mineral density; Osteoporosis; Plasma.
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1. Introduction

Osteoporosis is a prevalent problem of health and medical systems and in other words

Table 1. Descriptive characteristics of the study subjects (mean±SD).

	Patients (S)	Patients (M)	Patients (TP)	Controls
Femoral T-score	≤-1.7	-1.7<&≤-1	≤ -1	> -1
Age (yr)	57.98±11.49	49.64±8.23	55.24±11.19	47.62±10.43
BMI (Kg/m2)	25.05±3.28	26.29±3.55	25.46±3.40	27.64±4.53
Smokers	8	5	13	5
Number of children	3±2	3±3	3±2	2±2
Number of menopause	33	5	38	21
Plasma ascorbic acid (μM)	47.31±36.07	70.28±61.58	54.73±46.65	74.55±67.60

it is a silent epidemy. Bone fracture because of osteoporosis in elderly makes big problems and serious impairments and imposes high costs on families, society and health system. On the basis of data by endocrinology and metabolism research center at Tehran University of Medical Sciences, 20% of patients with spine or hip bone fracture have died during one year after the accident, and also 50% have encountered serious impairments and disabilities. There are 3 million people over the age of 65 in Iran and it is estimated that by 2020, it will be more than 10% of the population.

Up to now, the effects of age, sex, heredity and race on osteoporosis have been specified. The study of other factors including nutritional disorders, chronic hormonal and metabolic disease, menopause in women, use of corticosteroids, extended inactivity, smoking and alcohol consumption have been extensively conducted and new studies are in progress [1-5].

A number of dietary factors have been associated with bone mineral density (BMD) including calcium, vitamin D, vitamin C (ascorbic acid), caffeine, alcohol, etc. Ascorbic acid deficiency has been associated with decreased bone density in a few experimental animal studies [6, 7]. Also in human, several epidemiological and observational studies have reported correlation between dietary ascorbic acid intake and BMD [3-5, 8-10], but the relation of ascorbic acid and BMD has not been surveyed among a representative sample of the Iranian women. The aim of this study was the evaluation of plasma levels of ascorbic acid in osteoporotic patients compared to the control, and to investigate whether plasma levels of ascorbic acid were associated with BMD in Iranian women.

2. Materials and methods

2.1. Participants

In this cross-sectional study, participants were screened and selected among a total of approximately 1000 women that referred to bone mineral densitometry division of Jami Clinic in Tehran, Iran, between April and December 2006. Exclusion criteria included secondary osteoporosis, diseases of oxidative stress (diabetes, renal or hepatic insufficiency, cardio and cerebrovascular diseases, dementia and inflammatory diseases), malnutrition, hormone replacement therapy, use of antioxidant vitamins and antiresorptive drugs. Two hundred seventy women were selected and were asked to participate in the study. Finally, 200 women were enrolled. The project was approved by ethics committee of Tehran University of Medical Sciences.

Questionnaire included demographic variables (self reported age, body mass index (BMI)), history of diseases, nutritional status, smoking habit, functional status and disabilities, self reported fractures and usage of medicines. Questionnaire was filled by a trained interviewer. All participants were on free diet.

BMD of the femoral neck and lumbar spine was measured by dual x-ray absorptiometry. The participants underwent a fasting blood withdrawal in 10-ml heparinized tubes on the day of the bone scan and after centrifugation, plasma was divided in special vials, then temporarily was stored in liquid

Table 2. Plasma ascorbic acid levels and femoral neck T-score values in smokers and non-smokers.

Group	n	Ascorbic acid (µM)	Femoral neck T-score
Non-smokers	172	62.73±56.74	-0.62±1.32
Smokers	20	53.75±26.58	- 1.22±1.07

nitrogen and transferred to faculty of Pharmacy, Tehran University of Medical Sciences, for analysis.

2.2. Method

Ferric reducing ascorbic acid concentration (FRASC), a spectrophotometric assay, was used for measurement of ascorbic acid concentration. It is an acceptable alternative to HPLC method for measurement of plasma ascorbic acid [11].

2.2.1. Reagents and apparatus

Reagents were 300 mM acetate buffer, pH 3.6 [3.1 g sodium acetate trihydrate, plus 16 ml glacial acetic acid made up to 1 l with distilled water]; to give the working FRASC reagent, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) in 40 mM HCl; and 20 mM $FeCl_3.6H_2O$, in the ratio of 10:1:1). In FRASC, ascorbic acid in one sample of the pair of aliquots is selectively destroyed by ascorbate oxidase [EC 1.10.3.3 (Sigma)]. Therefore, ascorbate oxidase (40 µl of 4u/ml solution) was added to one of the plasma samples (100 µl), and water (40 µl) was added to another one. Then both of them were mixed with the FRASC working reagent. Finally the difference of absorbance, which is specifically due to oxidation of ascorbic acid, was measured with a spectrophotometer (UV visible, GBC Cintra 40, Victoria, Australia), at 593 nm. The substracted 0 to 4 min. absorbance changes are translated into µM of ascorbic acid by comparison with those of standard solutions of Fe (II) or ascorbic acid of the appropriate molar concentration [12].

2.2.2. Statistical analysis

Statistical analysis was performed using SPSS software version 12. Data are expressed as the mean±SD or as percentage. Descriptive statistics were conducted on all variables to evaluate range, variance, frequencies and normality of data. Demographic and clinical variables were compared by the X^2 test. Correlation analysis was carried out by means of the Spearman test. Analysis of covariance was performed to compare femoral Tscore as well as plasma levels of ascorbic acid between groups, with age and BMI as covariates. Statistical significance was defined as $p<0.05$.

3. Results

In this study, 200 participants were selected from the patients who referred to bone mineral densitometry division of Tehran Jami Clinic. Sum of 8 participants were excluded because of missing adequate data, so total numbers of subjects were 192. The T-score of both lumber spine (L_1-L_4) and femoral neck were measured in all participants. WHO (World Health Organization) has considered T-score>-1 as normal, -1>T-score>-1.7 as mild osteopenia, -1.7>Tscore>-2.5 as severe osteopenia and T-score< -2.5 as osteoporosis.

Control groups were selected based on both femur and spine T-scores, so control participants, n=76 (39.6%), were normal in both lumbar spine (L_1-L_4) and femoral neck (T-score ≥ -1).

Femur T-score was considered as criterion in selection of patient group. Seventy six subjects (39.6%) with T-score<-1 were known as total patient group (TP). TP was divided into mild patients (M; -1>T-score ≥ -1.7), and severe patients (S; -1.7> T-score; Table 1). A number of 40 patients (20.8%) of total participants entered neither patient group; because of femoral T-score>-1, nor control because of spinal T-score<-1.

Plasma ascorbic acid levels were compared between control and patient groups. Also Pearson correlation was used to examine the

Table 3. Correlation between plasma ascorbic acid levels and T-score values.

Group	Femoral neck T-score	n	P	Pearson correlation(r)
Patient (S)	≤-1.7	51	0. 519	+0.100
Patient (M)	-1.7< and ≤-1	25	0.745	-0.075
Patient (TP)	≤-1	76	0.108	+0.201
Control	>-1	76	0.282	+0.127
All subjects	-3.9 to 3.6	192	0.011	+0.192

associations of plasma ascorbic acid levels with bone mineral density in all participants, directly. In this way, the above mentioned 40 participants were included in the study, too. The demographic and clinical characteristics of groups are shown in Table 1.

No difference in menopause, nutritional habit, drugs and functional activities was found between groups. But there were significant differences in age, BMI and smoking.

Plasma ascorbic acid levels and femur T-score were entered into partial correlation model as continuous data with controlling for age and BMI (r=0.2253; p=0.023).

None of these interactive factors (age and BMI) had statistically significant associations with plasma ascorbic acid levels. Though 'r' was negative for all, but it is worth saying that there were lower plasma ascorbic acid levels in smokers than non-smokers (Table 2).

BMI was associated with femur mineral density (r=+0.388, p<0.01). Age was inversely associated with femur mineral density (r=-0.310, p<0.01). Although smoking habit was not associated with femur T-score, but femur mineral density was lower apparently in smokers than non-smokers (Table 2).

Mean±SD plasma levels of ascorbic acid was 74.55±67.60 M in control group, 54.73±46.65 μM in TP (T-score <-1), 47.31±36.07 μM in S patient group (T-score -1.7), and 70.28±61.58 μM in M patient group -1>T-score>-1.7), respectively. Mean plasma level of ascorbic acid was lower in patients than in control (p<0.05).

T-score was directly examined with plasma levels of ascorbic acid. The results are shown in Table 3. Femoral neck BMD, T-score, showed a positive and significant correlation with plasma levels of ascorbic acid in all participants. There were no significant correlation between plasma levels of ascorbic acid and femur T-score in control group or patients groups.

4. Discussion

We investigated the plasma levels of ascorbic acid as an antioxidant in osteoporotic women referring to Jami clinic, Tehran, Iran. Our results showed that: 1) plasma levels of ascorbic acid were lower in patients than in control; 2) this difference was higher between control and S patients group (T-score -1.7) with more acute disease, than M patients group (-1.7<T-score<-1) with milder disease; 3) femoral neck BMD, T-score, showed a positive and significant correlation with plasma levels of ascorbic acid in all subjects (r=+0.192, p = 0.01), but this relation was not found in control and patients groups, separately.

Some observational studies, seem to express a positive role although not consistently, for antioxidants in contrasting bone loss of women [3, 5, 13-15]. Dietary intake of ascorbic acid has proven to be protective against hip fracture in female smokers followed prospectively for up to 5 years [4]. Other epidemiologic studies have reported an association between diet, supplementary or serum levels of ascorbic acid and BMD or fractures in postmenopausal women [3, 5, 16, 17]. Maggio *et al* found ascorbic acid concentrations to be consistently lower in osteoporotic women group than in controls [18]. Some other studies do not give the same results. Simon and Hudes, by investigating relation of ascorbic acid with osteoporosis, reported: 1) among men, serum

ascorbic acid levels were associated in a nonlinear fashion with bone mineral density and self-reported fracture; 2) among premenopausal women, dietary ascorbic acid was associated with greater bone mineral density in a linear fashion; 3) among postmenopausal women with a history of smoking and estrogen use, serum ascorbic acid was associated with a marked decreased prevalence of self-reported fracture, and unexpectedly was also associated with lower bone mineral density among postmenopausal women without a history of either smoking or estrogen use [16].

In other study; Wolf *et al.*, by investigating women between ages of 50 and 80 years, did not report a significant relation between plasma antioxidants levels, such as ascorbic acid, and bone mineral density [19]. These studies present mixed results and they can hardly explain differences and interpretation, because studies varied in the measured exposure, the measured outcome, the measured site and the various confusing factors. In addition to cellular-molecular happenings in osteoporosis, production of free radicals increases bone cells and intracellular space by upsetting balance between osteoblasts and osteoclasts activities. Thus, bone loss increases and also bone formation decreases because of inhibitory effects of free radicals on osteoblastic differentiation [20]. One reason for bone mineral loss by smoking is exogenous free radicals and ascorbic acid reduction. Ascorbic acid is a potent antioxidant and protects body against free radicals [21], indeed, it is known as the terminal small-molecule antioxidant [22]. It is necessary in collagen biosynthesis that is main protein of bone matrix [23-25]. Other mechanisms may be related to the role of ascorbic acid in osteoblastic growth or in promoting calcium absorption.

In the current study, significant association between plasma levels of ascorbic acid and bone mineral density was observed, and the mean plasma levels of ascorbic acid in patients are lower than control group. On the other hand, femoral T-score had significant correlation with plasma ascorbic acid concentrations. So it will be interesting in more extensive studies to see whether changes in dietary or supplemental ascorbic acid use reflect changes in bone density; and researchers should consider whole food, food groups and whole antioxidants intake to solve this puzzle.

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