The Effect of vitamin C on bone mineral/mass density of menopausal women with equilibrated regime: A randomized clinical trial

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Abstract

In the present century, the decline of bone mineral density (BMD) and osteoporosis is a silent epidemic. The objective of the current study was to estimate the effect of vitamin C on BMD in menopausal women with balanced diet. Randomized, controlled clinical trial (RCT) was conducted as per WHO Registry Network. The study was done in Hafez Hospital of Shiraz University of Medical Sciences, Shiraz, Iran. 150 menopausal women suffering from osteoporosis were chosen. In randomized block design, the individuals were placed in the vitamin C (test) and placebo (control) groups. The test group received orally 1000 mg of vitamin C in two divided doses for 6 months and the control group similarly had placebo. Test parameter: Bone mineral density (BMD). Before treatment, considering some demographic specifications and the results of BMD were not different statistically (P>0.05) but there was significant difference in the age and obstetric history (P<0.05). There was a significant correlation between obstetric history with lumbar BMD (r=-295 and P=0.001) and femoral BMD (r=-0.226 and P=0.006). The average vertebral BMD did not indicate any significant difference between the vitamin C-treated group (0.02±0.05, P=0.001) and the controls (0.02±0.04, P<0.001). With respect to the findings of the study about the effects of vitamin C on BMD, antioxidant is suggested to add to the current treatments.

Keywords: Vitamin C, bone mineral density, menopause, balanced diet

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Introduction

Menopause is the most obvious sign of an aged woman and women pass about one third of their life after this period [1]. Menopause occurs between 46 and 58 years of age, with an average of 51.4 years. Approximately 90.0% of the women experience menopause during the first years of the sixth decade of their life [2]. During this period, some symptoms and complications occur due to the reduction of estrogen, the most important of which being osteopenia and osteoporosis [2]. These two cases as the silent epidemic of present era are types of metabolic diseases of bone and mineral metabolism. The rate of bone density increases since birth up to the age of 30-40 years, but the rate of removal is normally higher than creation, resulting in initiation of a process called “osteoporosis” [3]. This disease is epidemic among old individuals and World Health Organization (WHO) in 1999 announced it as the forth main enemy of human beings after cancer, Myocardial Infarction (MI), and stroke (CVA) [4]. Moreover, the male to female ratio of these ailments is about 3:1 because women experience lower bone density, longer life, and sudden subsidence of hormones during menopause [5]. In addition, the rate of reduction of bone density is very high during the first few years of menopause and loss of bone mass is accelerated during the first 5-10 years [6].

The results of a study on 256 menopausal women in Saudi Arabia in 2004 showed an increased risk of fracture due to osteoporosis among 74.0% of women at lumbar vertebral column and 59.0% at femur neck [7].

In the U.S., out of the 80.0% of the individuals who suffered from osteoporosis, 30.0% were women of 60 years old or below and 70.0% were women of 80 years old or above [8].
In Iran, Zolfaghari and et al. [9] reported that nearly 70.0% of the women and 50.0% of the men above 50 years old were suffering from bone mass reduction and/or osteoporosis. Besides, Ebrahimof and et al. [10] reported the rate of bone mass reduction and osteoporosis are of 55.6% and 33.3%, respectively among women above 50 years old and 69.2% and 7.7% among the men of similar age group. Additionally, Soltani and et al. [11] showed that the prevalence of osteoporosis was 27.8% among the women with the mean age of 53.4 years from Tehran.

Considering the importance of this epidemic disease among the aged population, two prevention methods, namely general (observing the regime and changing the life style) and specific (medicines), can be utilized [4]. This can be achieved through equilibrated dietary regime. This regime for adults includes 55.0% carbohydrates, 15.0% protein, and 30.0% fat [12]. Some materials, such as antioxidants especially vitamin C, which play a role in bone health have a more significant role in this regimen. This vitamin which is soluble in water is called “the king of antioxidants” and is good for natural growth, construction of bone cells, collagen, teeth, gum, and blood vessels, and is the stimulator of necessary phosphates. Besides, lack of vitamin C is accompanied by formation of unnatural bones [13].

Considering the cultural conditions of the Iranian women with respect to the type of their covering (lack of receiving vitamin D through sun shine), nutrition, low mobility, race, and reduction of the age of beginning of osteoporosis that could make them susceptible to osteoporosis and bone fracture, the researchers decided to study the effects of vitamin C on bone density of the menopausal women following an equilibrated regime. Using the study results, effective steps could be taken to recommend this supplement due to its significant effects on health.

**Methods**

The randomized double-blind clinical trial was conducted on the individuals referring to the Radiology Ward of Shiraz Hafez Hospital (2012-2013) for evaluating bone density for a period of 4 months. Among these women, 150 menopausal ones aging 45-60 years were selected through purposeful convenience sampling. The inclusion criteria of the study were confirmation of osteopenia or osteoporosis by a radiologist, not using hormones or medicines affecting bone metabolism during the last year, having experienced delivery, and having normal activity. The sample size of the study was calculated based on the study objectives and the previous studies conducted on the issue and considering the power of 80.0%, confidence level of 95.0%, and effect size of 20.0%. At first, the research objectives were explained to the study participants and written informed consents for taking part in the study were obtained. The samples were then divided into a case and a control group using block randomization. Afterwards, demographic information questionnaire was completed through interviewing the participants.

To have equilibrated regime (containing 50 - 60% carbohydrates, 12 - 20% protein, and 25.0% Fat), the study groups were trained for a week and homogenization was made according to the participants’ weight and height under the supervision of a nutrition specialist. The subjects were monitored for observation of the equilibrated regime and receiving a constant amount of vitamin C through using fruits. The participants were also provided with an educational pamphlet to continue the regime. The participants who were receiving vitamin E, D, and calcium previously or were required to use a supplement according to their present results of bone density also had to follow the equilibrated regime.

The study samples were given the drug or the placebo (in form of tablet), which was made by the faculty of pharmacy of Shiraz University of Medical Sciences. The drugs were exactly the same and were only differentiated by A and B stickers. The case group received 500 mg Vitamin C and the control group received the placebo twice daily for a period of 6 months. The drug packages were distributed monthly. Following this process, the study samples were contacted every week in order to ensure their consumption of the drugs and observation of the equilibrated regime.

In this study, the dose of vitamin C and the intervention period were selected according to other studies conducted on the issue. After 6 months, all the study participants were reevaluated for femur head and lumbar vertebra bone density. It is necessary to mention that in this study, the radiology technician, radiologist, and density measuring apparatus were constant. Bone density before and after the intervention was assessed using Dull X-ray Absorptiometry (DXA).DXA is known as the “golden criterion” and is a simple, painless, and non-invasive method which has the ability of measuring several regions, particularly vertebral column, pelvis, and forearm. The intensity of osteoporosis was classified based on the definition by WHO and the study samples were selected accordingly. After all, the data were entered into the SPSS statistical software (v. 16) and analyzed using descriptive statistics, independent t-test, paired t-test, Chi-square test, and ANOVA. Besides, $P<0.05$ was considered as statistically significant.

**Results**

This study was conducted on 150 menopausal women. Before the intervention, no significant difference was
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found between the two groups regarding the age of menopause, occupation, education level, existence of underlying diseases, and the results of primary bone density measurement \((P>0.05)\). However, a significant difference was observed between the two groups concerning age and midwifery history \((P<0.05)\) (Table 1). Considering daily activity, consumption of tobacco and alcohol, using supplements, doing hormone therapy, and/or previous use of vitamin C, the results showed a significant difference between the two groups regarding activity \((P=0.015)\) and consumption of calcium \((P=0.002)\), but no significant difference was observed with respect to other variables \((P>0.05)\).

After the intervention, the results revealed no significant relationship between occupation and daily activity and bone density \((P>0.05)\).

However, a significant reverse correlation was observed between the midwifery history and bone density of lumbar vertebra \((P<0.001, r=-0.295)\) and femur head \((P=0.006, r=-0.226)\) (Table 2).

Comparison of the mean of bone density index revealed no significant difference between the two groups before \((P=0.541)\) and after the intervention \((P=0.499)\). However, the results of paired t-test indicated that the mean of changes was significant in the vitamin C \((0.02\pm0.05, P=0.001)\) as well as in the control group \((0.02\pm0.04, P<0.001)\) (Table 3).

The findings of the current study revealed no significant difference between the two groups (independent t-test) and within groups (paired t-test) regarding femur head bone density before the intervention \((P>0.05)\). Also, the results of ANOVA showed no significant difference between the two groups in this regard after controlling age, midwifery history, and the previous BMD index \((P>0.05)\) (Table 4).

### Table 1. Comparison of mean and standard deviation of some variables in the case and control groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case Group</th>
<th>Control Group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>56.21±7.61</td>
<td>59.13±8.25</td>
<td>0.026</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>4.26±2.45</td>
<td>5.7±2.85</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of deliveries</td>
<td>3.8±2.06</td>
<td>5.25±2.53</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of children</td>
<td>3.58±1.92</td>
<td>5.10±3.93</td>
<td>0.003</td>
</tr>
<tr>
<td>Age at the first breastfeeding</td>
<td>20.88±4.92</td>
<td>19.10±5.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Number of breastfeeding</td>
<td>3.33±1.96</td>
<td>4.58±2.33</td>
<td>0.001</td>
</tr>
<tr>
<td>Menopausal age</td>
<td>47.82±5.45</td>
<td>47.69±7.48</td>
<td>0.901</td>
</tr>
</tbody>
</table>

### Table 2. The correlation between midwifery history and bone density in the case and control groups

<table>
<thead>
<tr>
<th>The index of osteoporosis</th>
<th>No. of pregnancies</th>
<th>No. of deliveries</th>
<th>No. of children</th>
<th>No. of breastfeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
<td>R</td>
</tr>
<tr>
<td>Lumbar BMD</td>
<td>&lt;0.001</td>
<td>-0.295</td>
<td>&lt;0.001</td>
<td>-0.295</td>
</tr>
<tr>
<td>Neck BMD</td>
<td>0.006</td>
<td>-0.226</td>
<td>0.007</td>
<td>-0.220</td>
</tr>
<tr>
<td></td>
<td>0.012</td>
<td>-0.206</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Comparison of the mean lumbar vertebra bone density in the two groups before and after the intervention

<table>
<thead>
<tr>
<th>Index</th>
<th>Time</th>
<th>Case group</th>
<th>Control group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>Before the intervention</td>
<td>0.81±0.11</td>
<td>0.79±0.11</td>
<td>0.541</td>
</tr>
<tr>
<td>After the intervention</td>
<td>0.83±0.11</td>
<td>0.81±0.10</td>
<td>0.499</td>
<td></td>
</tr>
<tr>
<td>Mean changes</td>
<td>0.02±0.05</td>
<td>0.02±0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. The mean changes of femur head bone density in the two groups before and after the intervention

<table>
<thead>
<tr>
<th>Index</th>
<th>Time</th>
<th>Case group</th>
<th>Control group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>Before the intervention</td>
<td>0.63±0.08</td>
<td>0.64±0.09</td>
<td>0.400</td>
</tr>
<tr>
<td>After the intervention</td>
<td>0.63±0.08</td>
<td>0.640,345±0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean changes</td>
<td>0.001±0.04</td>
<td>0.002±0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.537</td>
<td>0.655</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The results of this research showed no significant difference between the two groups before and after the intervention regarding increase in density at vertebral column and femur head. However, a significant difference was observed between the two groups’ mean density of vertebral bone before and after the intervention, showing a slight increase in density (Table 3& 4). This might be attributed to the positive effects of vitamin C.

Chui and et al. [14] conducted a study on menopausal women using four types of interventions (placebo, exercise and antioxidants, exercise and placebo, and antioxidants) for a period of six months. The results of that study showed that bone density of femur head remained constant in all the study groups. In addition, bone density at vertebral column decreased in the placebo group, but remained unchanged in the remaining groups. These results were somehow similar to those of the present study. Moreover, Leveille [15] and Morton and et al. [16] in their study prescribed daily consumption of 407 and 745 mg vitamin C, respectively for menopausal women for more than 10 years. The study results indicated an increase in the subjects’ bone density. The results of these studies are not in agreement with those of the present study even though 1000 mg vitamin C was prescribed in this study. This could be because of the short period of the intervention.

Rivas and et al. [17] assessed the relationship between the receiving pattern of vitamin C and minerals (Zinc-Selenium) and bone density before and after menopause. The study findings showed a positive relationship between using minerals and vitamin C and bone density in all the study groups.

The aforementioned study evaluated the effect of vitamin C together with two minerals on bone density, while only vitamin C was prescribed in the current study. Therefore, except for the similarity in ineffectiveness of vitamin C in bone density of vertebral column, the results are not consistent regarding the duration of the intervention, number of drugs, and their effects. Thus, one of the limitations of the present study seems to be its short period of vitamin C prescription since it requires more time to influence bone density.

Sahni and et al. [18] studied the effect of vitamin C on bone density and the risk of fracture in menopausal women for 17 years. According to the results, no bone density reduction was observed in the intervention group and the rate of fracture was also significantly lower compared to the control group. These results were in line with those of the present study concerning the lack of decrement in bone density and incidence of fracture in the intervention group. In our research, 5 women (6.2%) in the control group suffered from fracture at wrist and knee due to falling upon the ground, but no such problems were detected in the case group which might be due to consumption of vitamin C.

The results of our study also showed the simultaneous effect of equilibrated dietary regime and vitamin C consumption on bone density, particularly in the case group. In the same line, Shivani and et al. [19] investigated the effect of vitamin C and dietary regime on bone density in both women and men. Based on the results, a positive relationship was observed between receiving vitamin C and dietary regime (for four years) and femur neck bone density in non-smoker men ($P=0.04$), but no such relationship was observed in women ($P=0.09$). These results were similar to those of the present study, except that the samples of our study were all women and the period of the intervention was shorter.

The findings of the current study demonstrated that simultaneous prescription of vitamin C and calcium had a positive effect among the case group participants. Wang [20], Hall and Greendale [21], and Randi Wolf and et al.[22] in their studies assessed the effect of vitamin C, calcium, and hormone on bone density of menopausal women and observed a significant relationship between bone density of femur neck, pelvis, and vertebral column and vitamin C, calcium, and hormone therapy. However, no changes were detected in the vertebral column in Wang’s study.

The results of the above-mentioned studies are in agreement with those of our study because the case group received at least 500 mg calcium daily. This can also emphasize the positive effect of vitamin C in reabsorption of calcium.

The findings of the current study revealed a significant difference between the two groups’ midwifery history before the intervention ($P<0.05$) (Table 1). Moreover, a significant reverse relationship was observed between midwifery history and bone density reduction after the intervention (Table 2). These results were similar to those obtained by Allali [23] and Gur and et al. [24]. These researchers also reported a positive relationship between increase in the number of pregnancies and deliveries and reduction of bone density at vertebral column and femur region after menopause [25, 26].

Considering the association between bone density and age, age at first breastfeeding, and age at menopause, the results showed a positive relationship between bone density reduction and age ($P<0.001$), but not age at menopause and age at first breastfeeding ($P<0.05$). Similarly, the results of the studies by Blake [25] and Warming and et al. [26] showed density reduction at the...
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femur head at the beginning of menopause that continued after menopause and was associated with increase in age. Also, the results of the studies carried out by Carranza-Lira and Mera [27] and Matsushita and et al. [28] indicated no relationship between age at breastfeeding and bone density, which is consistent with the findings of the present study.

In addition to its large sample size (75 subjects in each group), this study was a double blind trial, making it a powerful study among epidemiological studies. However, this study had some limitations. In spite of the fact that the groups were homogenized to have equilibrated this study had some limitations. In spite of the fact that the groups were homogenized to have equilibrated regime, it was not possible to remove the supplements which were previously used by the individuals. Also, the short period of the intervention was another limitation of the study.

Conclusion

This study aimed to evaluate the effects of king of antioxidants on osteoporosis. Vitamin C plays an important role in reduction of osteoporosis, increase of bone density, and calcium absorption by its vast extra- and intra-cellular effects. Recently, more attention has been paid to the effect of this soluble antioxidant, leading to achievement of Noble Prize in medicine [29]. Due to the considerable effects of vitamin C on bone density particularly in menopausal women, the present study researchers assessed replacement or simultaneous prescription of this vitamin with other supplements. However, no acceptable results were obtained which could be due to various reasons, including personal differences in production of estrogen and androgen, BMI, and using effective medicines in bone density without informing the researcher. Therefore, further studies with longer intervention periods are recommended to be conducted in a surrounded environment to control the effect of confounders, such as uncontrolled consumption of drugs.

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References


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