Evaluation of Dietary Intakes and Urine Calcium in the Osteoporotic Postmenopausal Women

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Abstract: Numerous causes are posed as risk factors of low bone density. Adequate nutrition plays an important role in bone health. The aim of study is to determine relationship between low bone mass with dietary factors and calcium excretion in postmenopausal women. In a cross-sectional study, eighty postmenopausal women who referred to Rheumatology Clinics of Zahedan participated in the study. Based on bone mineral density (BMD) measurement at the femur neck, lumbar spine using Dual-Energy X-ray Absorptiometry (DEXA) method, 54 participants were osteoporotic (case group) and 26 were non-osteoporotic (control group). Diet evaluation was performed using 24-hr dietary recall questionnaire. One 24-hour urine specimen was collected for the measurement of calcium. The results demonstrated that the diet of patients contained inadequate calcium compared to Dietary Reference Intake (DRI) and control group (P<0.01). In 9 (16.7%) of cases, the Ca excretion (CE) was elevated in urine specimens, in particular, 8 (19.5%) of them were in the age 50 years and above. Among nutritional factors, exception of protein intake in the case group, no significant impact of the other dietary factors was observed on urine calcium excretion. A significant association between CE and protein intake was observed. The findings suggest that an insufficient intake of calcium and high protein intake are important agents in lowering BMD. Besides, the excess urinary calcium is also a major risk factor particularly in the osteoporotic women with age > 50 years and those receiving more than 1 gram of protein /kg (n=9, 100%). Although other nutrients did not show a significant difference, further investigation with larger populations is recommended.

Key words: Bone Mineral Density • Dietary Intakes • Urine Calcium Excretion • Postmenopausal Women

INTRODUCTION

Osteoporosis is one of the most common metabolic diseases which is found in the aged especially postmenopausal women [1-4]. Numerous causes are posed as risk factors of low bone mineral density. One of these factors is nutrients intake [5]. Physiological and psychological changes in menopause may lead to changes in appetite, resulting in dietary status of postmenopausal women may be impaired [3] (Kim). The role of nutrition in the prevention and pathogenesis of non communicable diseases has been described in earlier studies [1, 2, 4, 5-7]. Dietary patterns have great influence on body composition, including the amount of...
fat, protein and bone tissue [5, 6]. Some micronutrients especially minerals could affect skeletal balance [3, 5, 6, 8]. Several studies included the relationship between high intake of; animal proteins, phosphorous and sodium, alcohol, coffee and low intake of calcium and vitamin D absorption deficiency resulting from sunlight lack with low bone density in postmenopausal women [1-3, 6]. Alteration of these nutrients in the diet may lead to loss of bone calcium stores [5], calcium excretion [6, 9] and influence skeletal metabolism [10]. The excess urinary calcium “an indicative of hypercalcemia” [11, 12] has been suggested as one of the significant risk factors for low bone density [12].

The association between calcium intake and other dietary and non-dietary factors with calcium excretion has not been clearly explored [9]. With respect to high prevalence of osteoporosis and osteopenia in Iran [1, 13, 14], limited data on dietary intakes of osteoporotic postmenopausal women and importance of food intakes in the prevention of low bone density, the present study aimed to evaluate of dietary intakes and urinary calcium excretion in postmenopausal women.

**MATERIAL AND METHODS**

In a discretional cross-sectional study, 80 postmenopausal women (54.8±8 years) were selected from patients who came to rheumatology clinics of Zahedan“Center of Sistan and Baluchistan Province”, Iran, during September 2012 to June 2013. All participants were referred to densitometry center. Bone Mineral Density (BMD) measurement at the femur neck and lumbar spine (L2-L4) by Dual Energy X-ray Absorptiometry method (DEXA) (GE-Lunar Radiation Corporation, DPX MD + 73457 models, Madison, WI, USA) was performed. The results were defined according to T score and the diagnosis of osteoporosis / osteopenia was made based on WHO criteria [14]. Based on the results of BMD, participants were divided into two groups (with reduced bone density as osteoporosis and osteopenia (case group; n=54) and normal bone density (control group; n=26). The weight of subjects was measured at the time of the DXA scans with a clinical scale with a precision of 100 g. Participants were dressed in light clothes and did not wear shoes.

Women with medical history such as diabetes, thyroid, kidney disease, rheumatoid arthritis, or the recent medication use of Levothyroxine, furosemide, heparin, phenytoin, phenobarbital, vitaminK, ranitidine, calcium supplementation, vitamin D3 and corticosteroid, a history of hysterectomy or ovarietomy or smoking and alcohol consumption were excluded.

A structured questionnaire including demographic characteristics (such as age, weight, family history, medical and medication history, smoking habits, alcohol consumption and the levels of physical activity was fulfilled for each subject by a trained nutritionist. The physical activity level (PAL) was calculated by the ratio of total energy expenditure to basal energy expenditure. The PAL was determined with four categories: sedentary (PAL: 1.0-1.39), low active (PAL: 1.4-1.59), active (PAL: 1.6-1.89) and very active (PAL: 1.9-2.5) [15].

As well, dietary intakes were estimated using 24-hr dietary recall questionnaire [16, 17]. All of consumed food items were recorded on the two previous days. In order to avoid eventual differences between working and not-working days, the mean values of calories and nutrient intakes were measured on one weekday and one week-end. Mean nutrient intakes were compared with the Dietary Reference Intakes (DRI) [15, 18, 19]. The nutrients value of the diet was analyzed with a computer software program developed by the authors for analyzing of Iranian foods.

Then participants were referred to laboratory for measurement of urine calcium. Subjects were instructed on the technique of 24-hr urine collection. They were requested to discard the first specimen of the first day and then to collect all specimens for 24-hr, up to and including the first specimen of the next day. On the arrival at the laboratory, the volume of the 24-hr collections was recorded. Urine calcium was measured by atomic absorption spectrophotometer. The study complied with the code of ethics of the World Medical Association (Declaration of Helsinki). The protocol of study was approved by the ethic committee of the Zahedian University of Medical Sciences and informed consent was orally obtained from all patients and control group.

**Statistical Analysis:** Data were analyzed using SPSS software (Statistical Package for Social Sciences), version 18.0. All data were normally distributed and results expressed as means±S.D. Independent sample t-test and ANOVA tests were used for comparison of the groups as appropriate. The correlations between the variables were calculated by Pearson correlation test. The values of $p<0.05$ were considered significant.
Table 1: Demographic characteristics of the study population

<table>
<thead>
<tr>
<th>Group</th>
<th>Case (n=54)</th>
<th>Control (n=26)</th>
<th>Variables</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.2±8</td>
<td>52.7±8</td>
<td>0.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>66±10.7</td>
<td>82±12.4</td>
<td>&lt; 0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (Cm)</td>
<td>156±5.4</td>
<td>157±8.3</td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1±3.9</td>
<td>33.5±7.1</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD (T-score) Femur neck</td>
<td>-1.8±0.97</td>
<td>0.67±0.41</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>-2.27±1.2</td>
<td>0.85±0.54</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL</td>
<td>1.40±0.28</td>
<td>1.50±0.48</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunlight exposure</td>
<td>15.6±13</td>
<td>36.3±21.5</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine volume (cc)</td>
<td>1475±606</td>
<td>1421±563</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine creatinine (g/24 hr)</td>
<td>0.72±0.21</td>
<td>0.85±0.18</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine Ca mg/24hr Total</td>
<td>156.3±103.7</td>
<td>143.6±63.4</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n %)</td>
<td>45(83.3%)</td>
<td>26(100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n %)</td>
<td>9(16.7%)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PAL: Physical activity level; BMD: Bone mineral density Ca: calcium

Table 2: Urinary calcium excretion in relation to nutrients intake in the study population

<table>
<thead>
<tr>
<th>Groups</th>
<th>CE (mg/24 hr)</th>
<th>Cases</th>
<th>&lt;300</th>
<th>&gt;300</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹Daily dietary intakes</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>1595±495</td>
<td></td>
<td>1593±448</td>
<td>1605±371</td>
<td>1593±294.4</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>204.4±93.3</td>
<td></td>
<td>210±99.4</td>
<td>197.6±48</td>
<td>202±37.7</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>85±30.5</td>
<td></td>
<td>83.2±31.4</td>
<td>96±24.1</td>
<td>76.3±31.8</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>48.8±20.5</td>
<td></td>
<td>46.7±19.6</td>
<td>58±23.4</td>
<td>53.3±21</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>15±1.7</td>
<td></td>
<td>15.2±1.7</td>
<td>14.1±1.4</td>
<td>13±1.6</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>704±402¶</td>
<td></td>
<td>690±392¶</td>
<td>808±469</td>
<td>976.6±597</td>
</tr>
<tr>
<td>Phosphorous (mg)</td>
<td>1162±432</td>
<td></td>
<td>1125±410</td>
<td>1146±515</td>
<td>1284±531</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>1516±708</td>
<td></td>
<td>1400±634</td>
<td>1555±894</td>
<td>1684±663</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>2275±1260</td>
<td></td>
<td>2272±899</td>
<td>2293±910</td>
<td>2499±1037</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>336±263</td>
<td></td>
<td>338±273.4</td>
<td>326.4±220</td>
<td>341±222.4</td>
</tr>
<tr>
<td>Vitamin D (µg)</td>
<td>2.1±0.94</td>
<td></td>
<td>2.1±0.95</td>
<td>2.1±1.5</td>
<td>2.1±0.63</td>
</tr>
</tbody>
</table>

Values are presented as mean ±SD
CE: calcium excretion

* P<0.01: mean protein intake had a significant impact on calcium excretion.
¶ P<0.01: mean calcium and protein intakes vs. control
¹ Dietary intakes include intakes from food sources only

RESULTS

Eighty postmenopausal women aged 35-79 years were enrolled in the study. 54 (67.5%) of them had bone loss. The mean age of participants was 54.8±8 years. There was no significant difference in the mean age of patients with control group (P<0.05).

The mean weight was significantly lower in patients as compared to the control group (P<0.0001). The results of the DEXA scan based on the T-score values demonstrated that the mean bone density T-score was -1.8±0.97 in femur neck and -2.27±1.2 in Lumbar spine, respectively. There was no significant difference between the level of physical activity in both case and control groups. The patients had lower levels of sun light exposure as compared to the control group (P<0.01). In 9 (16.7%) of cases, the Ca excretion (CE) was elevated in urine specimens. There was significant difference between CE in cases and control group (P<0.01). A detailed description is shown in Table 1.

Table 2 indicates that the diet of osteoporotic women contained inadequate calcium compared to dietary reference intake (DRI) and control group (P<0.01). Between nutritional factors exception of calcium intake, in both of case and control groups no statistically significant difference was observed. In addition, high intake of protein had a significant impact on calcium excretion (P<0.01).

As shown in Table 3, the urinary calcium excretion tends to increase in the cases with age 50 years and above (n=8; 19.5%) and those taking >1 gram of protein/kg (n=9; 100%). Of osteoporotic women with hypercalciuria 4 (44.5%) had inadequate intake of calcium than Dietary Reference Intake (DRI) (P<0.05).

There was a positive correlation between dietary intakes of protein (r=0.89, P=0.02) with urinary calcium excretion only (Figure 1).
Table 3: Urinary calcium excretion in relation to changes of age, calcium and protein intakes in the study population

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE (mg/24 hr)</td>
<td>mean ±SD</td>
<td>n(%)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>137.2±79 (n=13)</td>
<td>12(92.3%)</td>
</tr>
<tr>
<td>≥50</td>
<td>*163±112 (n=41)</td>
<td>33(80.5%)</td>
</tr>
<tr>
<td>Daily dietary intakes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 800</td>
<td>¶167±124 (n=32)</td>
<td>28(62.2%)</td>
</tr>
<tr>
<td>800-1200</td>
<td>155±115(n=13)</td>
<td>10 (22.2%)</td>
</tr>
<tr>
<td>&gt;1200</td>
<td>154 ±98 (n=9)</td>
<td>7 (15.6%)</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.8</td>
<td>139±66 (n=15)</td>
<td>15(33.3%)</td>
</tr>
<tr>
<td>0.8-1</td>
<td>147.4±79 (n=9)</td>
<td>9 (20%)</td>
</tr>
<tr>
<td>&gt;1</td>
<td>¶219.2±156 (n=30)</td>
<td>21(46.7%)</td>
</tr>
</tbody>
</table>

CE: calcium excretion
* P<0.01 vs. the age of < 50 years and control group ; ¶ P<0.001 vs. other subgroups and P<0.01 vs. control group
NS: No significant

DISCUSSION

Several non-dietary and dietary factors affect bone density loss [20]. Considering to importance of dietary intakes in the prevention of bone density less, the effect of various dietary factors and urinary calcium excretion on bone mass loss was investigated in this article.

To date, the impact of diet and nutrition that modify the probability of bone loss has been assessed in several studies [4-6, 8, 9].

Calcium and vitamin D have been posed as one of the most important micronutrients affecting the prevention of low bone mass at all ages [1, 21].

Numerous studies have shown a significant correlation between calcium intake and low bone mass [21-23]. Rizzol et al. [23] reported that the low amount of calcium intake is one of the most important risk factors of low BMD in premenopausal women. The other research showed that adequate intake of calcium increase the positive effect of estrogen on bone mass [21]. Some studies did not also find significant association between intake of calcium and bone loss [24, 25, 26].

At present study, the findings of daily dietary intakes showed that the mean calcium intake was found to be significantly decreased in patients with low BMD as compared to the normal group and DRI. Based on the standards set by the Food and Nutrition Board Dietary Reference Intakes [15, 19], only 9 (16.7%) of the osteoporotic women had an adequate intake and 32 (59.3%) consumed less than 800 mg of calcium (data not shown). There is the wide range of daily calcium recommendations which varies with age. The importance of high intake of calcium that can be imposed into bones and compensate the loss of calcium has been debated for a long time and there is still no clear guidelines about how much calcium is needed to prevent osteoporosis [27]. However, the maximum daily amount suggested by National Academy of Sciences is 1200mg per day in those >50 years of age [15, 19]. As people age, the bones lose calcium, in particular, women are at higher risk of fractures and osteoporosis than men [25]. The reduction of estrogen secretion after menopause can lead to low bone
density, resulting in severe osteoporosis [3]. Thus, women especially should take high calcium to maintain bone health with aging [7, 25, 27], in particular, when dietary vitamin D intake is low [7]. If its not possible to intake enough calcium from the diet, calcium supplementation maybe needed to maximize absorption [21] and to prevent bone loss [28].

Dietary intake of vitamin D has more protective effects for normal bone metabolism. Adequate vitamin D is also essential for enhanced absorption of calcium, in particular, at low- to moderate calcium intakes [7, 21, 25]. Food and Nutrition Board set the adequate daily intake at 15 µg for women < 70 yr of age and 20 µg for those > 70 yr of age [15, 19]. However, yet vitamin D inadequacy is common especially between aging. In this study, all of participants had also very low intake of vitamin D. But, there was no significant difference between the case and control groups. Since there are small amounts of vitamin D in some foods such as fish, eggs and butter, it is difficult to obtain enough vitamin D from diet alone. Thus, people who are at high risk of vitamin D deficiency may need to the supplemental vitamin D [25, 29].

At present study, patients had lower levels of sunlight exposure compared to the control group. Reasonable balance of exposure to sunlight can ensure that people are not at risk of vitamin D deficiency [29, 30]. Feskanish et al. [25] reported that the adequate intake of vitamin D decrease risk of hip fracture, while Michaelsson et al. [26] did not show the association between dietary calcium and vitamin D intake and fracture in the Swedish women aged 50–85 year.

Another finding in the present study is the significant difference between the control and the case group with regard to protein intake. Increased intake of protein was shown as a probable risk factor in this study.

The role of dietary protein in bone health has been controversial [31]. Several studies have pointed that anabolic effect of amino acids on bone is reduced in the elderly, especially, in women after menopause [9, 32]. Thus, increased protein intake may further protect elderly people against hip fracture [30] and increase bone mineral density [31-33]. As well, Gaffney-Stomberg et al. [32] suggested that Recommended Dietary Allowance (RDA) of dietary protein (0.8 g/kg per day for adults aged 19 and above) should be increased to 1.0-1.2 g/kg /day in the aged people to maximize its anabolic effect on bone. While other studies have reported that dietary proteins have a moderate beneficial effect on bone density [9] and high intake leads to hypercalcuiuria [5] and loss of bone calcium stores [9].

The findings of the Framingham Osteoporosis Study demonstrated that protein intake less than 46 g/ day recommended for adults increased risk of hip fractures and bone loss [30].

Hannan et al. [30] suggested that in addition to the high protein intake, regular exercise to build stronger muscles and better balance protect the seniors against hip fracture. In addition to the risk factors mentioned above, body weight is also embraced in the risk assessments for predicting fracture risk in postmenopausal women [35, 36]. We previously reported the relationship between weight and BMD in this population [37].

Urinary calcium excretion is also one of the significant risk factors for bone loss, which was assessed in this study. It can be used to assess hypercalcuiuria [11]. Urine calcium level “as a fraction of dietary intake [11]” was measured to estimate the calcium intake and the impact of other dietary factors on calcium excretion when possible.

The normal daily excretion of calcium for women consuming an average daily intake of 600 to 800 mg of calcium per day is 20 to 275 mg/ 24 hr [38]. The calcium excretion (CE) more than 300 mg/24 hr is considered as hypercalcuiuria [12].

Our results showed that the mean calcium excretion in the case and the control groups were significantly different (P<0.01). CE tended to increase in 9 (16.7%) of osteoporotic women, in particular, 8 (19.5%) of them were in the age 50 years and above. Older age is one of the non-dietary factors affecting urinary calcium excretion [4, 21]. In the elderly, the formation of skeletal mass is lower than resorption, which leads to age-related bone loss [9, 28, 39]. Increased calcium excretion in aging may be secondary led to severe skeletal resorption [39]. Unlike of our study, Taylor et al. [20] reported that aging inversely associated with urinary calcium excretion. However, there are few studies reported on this issue.

Among nutritional factors, exception of calcium and protein intakes in the case group, no significant impact of the other dietary factors was observed on urine calcium excretion in the present study.

We assessed the effect of three levels of two important nutritional factors including: calcium intake (< 800, 800-1200,> 1200 mg/day) [15, 19] and dietary protein (< 0.8, 0.8-1, > 1 g/kg/day) [17, 18] on the hypercalcuiuria.

The findings of study showed that calcium excretion (CE) tended to increase in those taking less than 800 mg of calcium (n=4; 44.5%). This finding is inconsistent with Taylor et al. [20] and Pak et al. [39] results.
However, it is important to note that, because about half of serum calcium is bound to proteins, abnormal serum calcium levels may occur secondary to serum proteins disorders[28], although, the levels of serum calcium and protein were not measured in this study.

Several studies have reported that calcium excretion (CE) is also influenced by protein intake [6, 9, 14, 20, 40, 41]. However, results of epidemiologic studies are inconsistent. Dietary proteins affect acid-base balance and interact with dietary calcium and phosphorous [9, 41]. The harmful effect of high protein diet (especially animal sources) on bone loss has been thought to be related to high endogenous acid load from the sulfur-containing amino acids in methionine and cysteine which have probably demineralizing effect and increases osteoclast-mediated bone resorption [6, 9]. The impact of protein on calcium loss may be compensated with increased intestinal absorption of calcium, unless calcium intakes are quite low [42]. A study showed that dietary protein is a more important regulator of calcium excretion than dietary calcium intake [43]. In our study, the assessment of three levels of dietary protein intake showed that urinary calcium was just seen in subjects receiving the high protein (> 1 g/kg) (n=9; 100%). A significant association between the excretion of calcium and protein intake was found in this study. This finding is consistent with the results of previous studies which found a positive correlation between animal protein intake with calciuria [10, 30, 31, 34, 40], although the source of dietary protein was not tracked in this study.

CONCLUSIONS

The findings showed that an insufficient intake of calcium and high protein intake are important agents in lowering BMD. Between the risk factors of hypercalciuria in the studied samples, a significant difference found between those with age > 50 years and high dietary protein intake. Although other nutrients did not show a significant difference, further investigation with larger populations is recommended.

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