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The Dietary, Serum and Urine Analysis of Boron and Micronutrients in Postmenopausal Women

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ABSTRACT

Objective: Boron is a nutritionally important trace element that interacts with other micronutrients. Boron plays a critical role in bone mineralization and metabolism. In the present study, the association between boron and micronutrients related to bone metabolism was analysed in postmenopausal women.

Methods: In a prospective cohort study in 40 postmenopausal women 24-hour urine and blood samples were collected for sodium, phosphorus, calcium, magnesium, and boron. Daily food consumption, bone mineral density, and Fracture risk assessment tool scores were recorded.

Results: The mean age was 53.2 ± 5.9 years. Dietary habits revealed insufficient dietary fiber and excessive dietary sodium. The serum and urine boron levels were 26.80 µg/L and 21.22 µg/day, respectively. Urine boron levels were lower in the osteoporosis group ($p = 0.66$). A negative correlation between urine Na and boron was detected ($p < 0.001$). Urinary Na and Ca are negatively correlated with Fracture risk assessment tool scores ($p = 0.010$, $p = 0.019$, respectively).

Conclusion: The low urinary boron levels in our participants might be due to increased Na excretion due to excessive consumption of Na. Therefore, consulting postmenopausal women about their dietary habits is of concern. Further understanding of the role of boron in bone metabolism will help to accomplish new treatment strategies for osteoporosis and standardization of boron supplementation.

Keywords: Boron, Mineral Metabolism, Menopause, Bone

1. INTRODUCTION

Boron is a nutritionally important trace element and daily boron intake differs according to personal food consumption. Vegetables and fruits are the primary sources of boron, whereas the highest amount of boron can be found in leafy greens. Absorption of boron can easily happen in the gastrointestinal tract and excretion mostly happens with the urine. Despite high amounts of boron intake, there is no significant change in plasma levels, as plasma boron levels are generally kept steady-state by homeostatic mechanisms.

Accumulating evidence in the literature indicates that boron plays a role in mineral and estrogen metabolism (1). In menopausal women, boron deprivation might be associated with menopausal symptoms and bone health. Therefore, research about the effect of boron supplementation on menopausal women has been of interest (2). In the study of Nielsen and Penland et al., women experiencing discomforts associated with menopause were given boron supplementation (3). The patients in this study did not have symptoms of relief in terms of hot flashes and night sweats, but serum 17b-estradiol concentrations were increased (3). As a result of their study, the authors concluded that boron possibly affects hormone processes in humans at the cell membrane level (3).

The major impact of boron in human metabolism is on bone health. The distribution of boron in bone is greater than the other tissues. The previously documented relationships of boron with micronutrients like vitamin D, calcium (Ca), magnesium (Mg), and phosphorus (P) indicate a strongly possible role of boron on bone health (4,5). Boron, directly or through the mechanism of other minerals and micronutrients can affect the development of bone formation. In animal models, boron has an effect on the enhancement of cell maturation and growth for long bones (4,5). Postmenopausal women who received boron supplementation have higher Ca absorption and lower urinary magnesium excretion (6,7). Considering evidences about the role of boron on bone health gathered from in vivo and vitro studies, this study was designed to explore the boron levels and other

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micronutrients like Mg, Ca, P, and vitamin D, related to bone metabolism in postmenopausal women.

2. METHODS

Our study was prospectively carried out in a university-affiliated hospital. All patients were informed and consents were collected from all participants. Approval was obtained from local ethical committee (200620117/06.2017). Candidates were selected from the women who admitted to gynecology clinic fulfilling the inclusion criteria during the study period.

Inclusion criteria was being in menopause that was defined as at least one year no menstrual bleeding, serum follicle-stimulating hormone (FSH) level >40 mIU/mL, serum estradiol (E2) level <25 pg/mL. All of the participants of the study were over 40 years of age and all were nonsmokers. Weight (kg) / height (m^2) was used to calculate the body mass index (BMI). The biochemical blood analyses of the participants, including serum creatinine levels, were normal. Patients' glomerular filtration rate (GFR) were measured by the modification of diet in renal disease (MDRD) equation (8).

Exclusion criteria were patients diagnosed with hypogonadism, hyperparathyroidism, thyroid dysfunction, chronic liver disease, chronic renal disease, malabsorption, malnutrition, diabetes mellitus, pregnancy, Cushing syndrome or any other diseases which can cause secondary osteoporosis or under osteoporosis treatment or receiving hormone therapy. Furthermore, patients, having any history of antiepileptic, corticosteroid, anticoagulant drug use, alcohol consumption and/or GFR <60 ml/min/1.73 m^2, were excluded from the study.

2.1. Serum markers

Biochemical measurements were taken at least 12 hours overnight fasting. FSH, E2, alkaline phosphatase (ALP), parathyroid hormone (PTH), calcitonin, 25 hydroxyvitamin D3 (25-OH D3), serum electrolyte and mineral levels (Ca, P, Zinc (Zn), Mg, Sodium (Na)) were measured. Having vitamin D level less than 20 ng/ml was defined as deficiency.

2.2. Serum and urine boron analysis

Non-heparinized tubes were used to collect blood samples for boron from the antecubital vein. To estimate the dietary boron intake, 24-hour urine samples after the first urine in the morning were collected. Boron levels were measured using Inductively Coupled Plasma – Mass Spectrometer (ICP-MS, 7500 cx, Agilent Technologies, Inc.). For the boron analyses by ICP-MS, the intra-assay coefficient of the variation was 1.66% and inter-assay coefficient of variation was 3.26%.

2.3. Bone mineral density measurement

Bone mineral density (BMD), expressed in g/cm^2, was measured at the lumbar spine (L1-L4) and femoral neck, the greater trochanter, Ward's triangle. Dual Energy X-ray Absorptiometry (DEXA, GE Healthcare DPX Duo) was used to measure BMD. Femoral neck BMD was measured perpendicular to the femoral midline at the narrowest point of the femoral neck. Spine BMD was found from the mean of four lumbar vertebrae (L1-L4) and measured in the anteroposterior view. The same radiology technician administered all the bone scans to eliminate discrepancies. Definitions of osteoporosis based on BMD measurements provided by The World Health Organization (WHO) were used to define normal, osteopenia and osteoporosis groups (9).

2.4. Fracture risk assessment tool (FRAX)

While evaluating the 10-year probability of hip fracture risk and major fracture risk, fracture risk assessment tool (FRAX®) was used. FRAX integrates age, gender, height, weight, risk factors and BMD of femoral neck in g/cm^2 to calculate the risk as a percent (10,11).

2.5. 24-hour urine analysis

24-hour urine samples were collected. The first urine in the morning was discarded and the urine on that day was collected for 24-hours including the first urine of the following day. Na, P, Ca and Mg levels were measured in 24-hour urine samples.

2.6. Dietary analysis

In order to evaluate food consumption frequency, 54 kinds of food and dietary habits were recorded. The researcher with an interviewer filled in the form. Individual food records were filled in by the patients participating in the study for determining personal food consumption (twice in the weekdays and once in the weekend, 3 days in total). According to Dietary Reference Intake (DRI) dietary fiber and Na levels were categorized as sufficient, insufficient and excessive consumption (12-14).

IBM Statistical Package for the Social Sciences (SPSS) version 11.5 was used for the statistical analysis. The Shapiro-Wilk test was used to evaluate the distribution of continuous variables. Descriptive statistics for continuous variables were presented as mean ± standard deviation or median (minimum-maximum) and for categorical variables were shown as the number of patients and percentage (%).

The significance of the difference based on the averages was assessed by Student's T test if two independent groups were present and one-way analysis of variance (One-Way ANOVA) if more groups were present. Likewise, the significance of the difference based on the median was analyzed by the Mann-Whitney U test when two independent groups were present and the Kruskal-Wallis test when more groups were present. If one-way analysis of variance or Kruskal-Wallis test statistical results were considered important, post hoc Tukey HSD or Conover non-parametric multiple comparison

tests were used to determine the conditions leading to the difference. Nominal variables were evaluated using Pearson's chi-square test. $p < 0.05$ was considered to be statistically significant.

3. RESULTS

The data from forty menopausal women were eligible at the end of the study period. The mean age and menopause age of the patients were 53.2 ± 5.9 and 48.7 ± 3.54 years, respectively. The median value for menopause duration was 3.0 years (ranging from 1 to 19 years). Levels of FSH, E2, ALP, PTH, calcitonin, and 25-OH D3 were 60 (40 – 177) mIU/mL, 15 (5-25) mIU/mL, 77±22 U/L, 47 (26-119) pg/mL, 3.5±0.7 pg/mL, 15(5-78) mg/L, respectively. The serum levels of Ca, P, Zn, Mg, Na were within normal ranges.

Among the participants of this study 70% (n=28) had vitamin D deficiency. No significant difference was observed in serum parameters when the patients with vitamin D deficiency were compared to cases with normal vitamin D results, except PTH. The PTH levels were significantly higher in cases with vitamin D deficiency when compared to cases with normal Vitamin vitamin D levels (61 vs 45 pg/ml, p = 0.043, respectively).

Results of 24-hour urine analysis for Na, P, Ca, and Mg were 107 (23-278) mEq/day, 718 ± 220 mg/day, 139±76 mg/day, 95±27 mg/day, respectively. The urinary Na excretion was significantly higher in cases with vitamin D deficiency than in cases with normal vitamin D levels (114 vs 64 mEq/day, p = 0.004, respectively).

According to DRI the adequacy of diet; all patients have insufficient consumption of dietary fiber (16.27 g/d) and excessive consumption of dietary Na (5120.79 mg/d). The daily consumption of Ca, Mg, P, Na, K, Zn, and Cu were compared between osteoporosis, osteopenia and normal BMD groups. No significant difference was found between the groups regarding the consumption of these micronutrients.

The median value for 24-hours urine boron levels and serum boron were 21.22 µg/day (0.02122 ppm, range: 3.65-56.11 µg/day) and 26.80 µg/L (0.0268 ppm, range: 8.80-66.40 µg/L), respectively. Neither serum boron levels nor urinary boron levels were found to be correlated with any of the serum parameters, including E2 (p = 0.38, p = 0.46, respectively).

Among the 40 patients, 12 (30%) had osteoporosis, 13 (32.5%) had osteopenia and 15 (37.5%) had normal BMD according to WHO criteria. The patients with osteoporosis tend to be older (p = 0.009). The BMI of the groups were similar in osteoporosis, osteopenia and normal BMD groups(p = 0.33). In addition, osteoporosis group's age and duration of menopause were significantly higher when compared with osteopenia and normal BMD groups (p = 0.027 and p = 0.026, respectively) (Table 1).

Table 1. *Comparison of age, menopause age and duration of menopause in the study group.*

	Osteoporosis (n=12) (%30)	Osteopenia (n=13) (%32.5)	Normal BMD (n=15) (%37.5)	p
Age (year) (mean±SD)	57.4±6.9	51.8±2.8	51.0±5.5	0.009*
BMI (kg/m2) (mean±SD)	29.1± 3.8	29.1±5.3	31.5± 5.0	0.33
Menopause age (year) (mean±SD)	50.6±3.8	48.9±2.8	47.0±3.2	0.027*
Duration of menopause (year) (median, min-max)	5.0(3.0-19.0)	3.0(1.0-5.00)	2.0(1.0-8.0)	0.026*

BMD: Bone mineral density, SD:Standart deviation.

**p values <0.05*

When osteoporosis, osteopenia and normal BMD groups were compared, although not significant, osteoporosis group's urine boron levels were lower when compared to osteopenia and normal BMD groups (p = 0.66) (Table 2).

Table 2*. Comparison of serum boron level and urine boron level in the study group.*

(median, min-max)	Osteoporosis (n=12) (%30)	Osteopenia (n=13) (%32.5)	Normal BMD (n=15) (%37.5)	p
Serum boron level (µg/L)	28.15 (14.80-45.30)	27.30 (11.90-66.40)	26.50 (8.80-40.80)	0.66
Urine boron level (µg/L)	16.835 (8.17-56.11)	27.00 (3.66-48.88)	21.66 (4.20-52.20)	0.834

BMD: Bone mineral density.

**p values <0.05*

Based on the results, there were no statistical significance between BMD parameters (Lumbar and Femur T scores) and serum or urine boron levels (p = 0.35, p = 0.60; p = 0.6, p = 0.41, respectively).

According to FRAX scores, 10-year probabilities for major osteoporotic fracture risks of all participants were lower than the defined threshold (20%). Only one case had ten-year probability of hip fracture risk (>3%) in the study group. The correlation analysis of 24-hour urine results of elements with FRAX scores showed that 24-hour urinary excretion of Na was negatively correlated with major osteoporotic fracture risk scores and also with hip fracture risk scores (r = – 0.401, p = 0.010 and r = – 0.0336, p = 0.034; respectively). The 24-hour Ca excretion was negatively associated with FRAX major osteoporotic fracture risk scores (r = – 0.370, p = 0.019).

In the correlation analysis of serum electrolyte and mineral levels, a negative correlation between serum magnesium level, serum boron levels and 24-hour urine boron levels were found statistically significant. Furthermore, a positive correlation between serum zinc level and serum boron levels and 24-hour urine boron levels were determined (Table 3).

Table 3. *The results of the correlation analysis of serum and urine boron with serum electrolytes, minerals and vitamin D.*

	Serum Boron Level		Urine Boron Level	
	r	p	r	p
Calcium	-0.061	0.71	-0.093	0.56
Phosphor	0.076	0.64	0.099	0.54
Zinc	0.534	<0.001*	0.365	0.020*
Magnesium	-0.349	0.027*	-0.353	0.026*
Sodium	0.149	0.36	-0.091	0.58
PTH	-0.117	-0.47	-0.301	0.058
Calcitonin	-0.069	0.67	-0.040	0.81
ALP	-0.017	0.91	0.009	0.95
25-OH-D3	-0.139	0.39	-0.033	0.84

ALP: alkaline phosphatase, PTH: parathyroid hormone, 25-OH-D3: 25-Hydroxyvitamin D3
**p values <0.05*

In the correlation analysis of 24-hour urine electrolytes (Na, Ca, P, Mg), with serum boron and 24-hour urine boron levels, a negative correlation between 24-hour urine Na and 24-hour urine boron level was found ($r = -0.528$, $p < 0.001$). Additionally, a positive correlation between 24-hour urine phosphorus and 24-hour urine boron level were also detected ($r = 0.545$, $p < 0.001$) (Table 4).

Table 4. *The results of the correlation analysis of serum and urine boron with 24-hour urine parameters.*

	Serum Level Boron		Urine Boron Level	
	r	p	r	p
24-hour urine Na (mEq/day)	-0.103	0.53	-0.53	<0.001*
24-hour urine Ca (mg/day)	-0.035	0.83	0.095	0.56
24-hour urine P (mg/day)	0.107	0.51	0.54	<0.001*
24-hour urine Mg (mg/day)	0.119	0.46	0.30	0.061

**p values <0.05*

4. DISCUSSION

The previously reported reference range for median urine boron value was 647 (282-2072) µg/L (15). In our study, the mean urinary boron excretion was much less than this value (21.22 µg daily (range, 3.65-56.11 µg/day)). Our findings revealed that urinary excretion of boron was negatively correlated with Na excretion. The low urinary boron levels in our participants might be due to increased Na excretion related to excessive consumption of Na. Therefore, consulting postmenopausal women about their dietary habits is of concern.

Although the underlying mechanism for skeletal mineralization has not fully been understood yet, it can be stated that an alkaline environment increases bone density and prevents osteoporosis that makes it a prerequisite for bone mineralization (16). Na(+)/H(+) exchanger proteins play an important role in intracellular ion homeostasis in osteoblasts (17). The kidney buffers the acidic medium and the excretion of Na with Na / K – ATPase pump helps to organize the structure of the bone mineral medium and avoid bone resorption by converting acidic environment to alkaline medium (18). In this context, the hypothesis that urinary Na excretion was negatively associated with bone mass and the risk of osteoporosis was tested by Park et al. (19). The authors reported that in premenopausal and postmenopausal women, urinary Na excretion was negatively related to bone mineral content (BMC) and bone mineral density (BMD) (20). In our study, the negative correlation between urinary NA excretion and FRAX scores supported this data. Moreover, the food records of our patients showed excessive consumption of dietary Na. We can conclude that excessive consumption of salt could be a potential risk factor for bone fracture, as suggested by others (20).

In bone mineralization, calcium phosphate crystals secure bone hardness. The gut, bone, and kidney hold important transport mechanisms for P and Ca, which are regulated by PTH and 25-OH D3. P and Ca metabolism are intimately interrelated (21). Moreover, Mg determines the metabolic fate of Ca and its deficiency causes urinary excretion and accumulation of Ca in soft tissues (18,22). Accordingly, clinically neither can be considered in isolation. The interest in this study was boron and there was in vivo and in vitro evidence that boron was interrelated with these mechanisms at different stages. Boron supplementation was associated with increased Ca absorption and lower urinary Mg excretion (6,7). In animal nutrition models, the presence of moderate amounts of vitamin D in boron and Mg containing diets showed that in a magnesium-deficient diet but supplemented with boron, the animals were able to maintain an adequate rate of growth, independent of vitamin D presence (4,5). In chickens, a diet deficient in vitamin D but supplemented with boron shows increased 25-OH D3 levels (6). Our results, with a study group of which 70% suffering from vitamin D deficiency, showed a positive correlation between urinary P excretion and urinary boron excretion. Moreover, serum levels of Mg were positively correlated with serum and urine levels of boron. More information about boron helps better understanding of mechanisms regarding bone metabolism.

The limited number of participants and lacking dietary boron intake were limitations of this study. In the comparison of healthy and osteopenia and osteoporosis groups in terms of serum boron level, with an error rate of 5% the power of our study is 32%. The low number of participants also disabled us to draw strong conclusions. In conclusion, large population-based studies are needed to confirm the relation between micronutrients and boron in postmenopausal women.

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Conflicts of interest

The authors report no conflict of interest.

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