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Original Article

Correlation between bone mineral density and serum trace element contents of elderly males in Beijing urban area

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Abstract: Trace element levels are associated with the incidence of osteoporotic fractures, but related mechanisms remain unknown. Trace elements may interfere with growth, development and maintenance of bones. Therefore, we investigated whether plasma trace element levels are associated with bone mineral density in elderly males in Beijing. After epidemiologically investigating 91 elderly males with age ranging from 50 years to 80 years, we obtained a total of 30 healthy (group 1), 31 osteopoenic (group 2) and 30 osteoporotic (group 3) subjects. Blood was collected, and serum concentrations of trace elements were detected. Elderly males in the three groups were carefully matched in terms of body mass index. Iron, manganese, zinc, copper, selenium, cadmium and lead were analysed by inductively coupled plasma-mass spectrometry. Bone mineral density (BMD) was measured by QDR-2000 dual-energy X-ray absorptiometry. Correlation between BMD and serum element contents was analysed using SPSS16.0. The plasma levels of manganese, zinc, copper, selenium and lead were similar in all of the groups ($P>0.05$). Cadmium was significantly and negatively correlated with BMD of the lumbar vertebrae ($P<0.05$). Moreover, cadmium and iron contents significantly differed in osteoporotic and healthy groups. These elements may directly and correlatively affect BMD in elderly males. Many trace elements may directly and correlatively influence BMD. Future studies should be conducted to evaluate serum and bone levels of these trace elements to determine the relationship of these trace elements with osteoporosis.

Keywords: Osteoporosis, bone mineral density, element

Introduction

Osteoporosis, a common bone disorder, is prevalent in postmenopausal females and elderly males. Osteoporosis is characterised by reduced bone density and altered bone microarchitecture. The primary complication of osteoporosis is fracture, which occurs at almost any site but most commonly on the hip, the vertebral spine and the wrist [1]. Approximately 10% of fractures affect the hip in age group of 60 years to 80 years; this proportion has increased to 41% in the age group of >80 years. An osteoporosis epidemiology study conducted in Dubbo (New South Wales, Australia) has shown that approximately one-half of hip fractures occur before males reach 80 years of age and two-thirds of the same disease occurs before females reach 85 years of age [2]. The incidence of distal forearm, hip and total fractures exponentially increase in both genders as these individuals age [3]. In Europe, osteoporotic fractures account for higher disability adjusted life year (DALY) lost than common cancers except lung cancer [4]. In another research, the economic burden of osteoporotic fractures in Europe will possibly increase from €36.3 billion in 2000 to €76.8 billion in 2050 [5]. The pathogenesis of osteoporosis may also be associated with trace elements [6, 7]. For instance, studies [8] have investigated the relationship between postmenopausal osteoporosis and trace ele-

ments. Trace mineral supplements with or without calcium elicit beneficial effects on the bone density of postmenopausal females [4]. Despite this association, the correlation between trace elements and BMD in males has been rarely analysed. Likewise, individual studies on trace elements, including selenium, zinc and copper, have demonstrated that deficiency in any of these trace elements can increase the risk of bone resorption by inhibiting bone growth; thus, these elements may play a role in the onset and the progression of osteoporosis [9]. However, the association between trace element status and osteoporosis in males has not been investigated. The relationship between elements and bone mineral density (BMD) should be elucidated to provide significant evidence for osteoporosis diagnosis and intervention. Thus, we investigated 91 elderly males in Beijing to further analyse trace element levels. In particular, serum concentrations of trace elements, including iron, manganese, zinc, copper, selenium, cadmium and lead, were determined. BMD was also detected. Furthermore, correlation between these trace elements and BMD was analysed.

Materials and methos

Subject

In this case-control study, 91 elderly men were divided into three groups based on their BMDs: healthy (group 1, n=30, T score <-1.0); osteopenic (group 2, n=31, -1.0< T score <-2.5); and osteoporotic (group 3, n=30, T score >-2.5). The subjects were carefully matched on the basis of their body mass indices (BMIs). Subjects were included in the study if the following criteria were satisfied: male subjects aged 50 years to 80 years of Chinese Han nationality; subjects who have lived in Beijing urban area for more than three years; subjects who do not suffer from diseases that may influence bone metabolism, severe chronic diseases requiring long-term therapy and diseases that can influence the secretion of male sex hormones; and subjects who do not have history of hormonal drug intake and osteoporosis treatment six months before our study was conducted. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the 309th Hospital of PLA. Written informed consent was obtained from all participants.

Density determination

Osteoporosis was diagnosed on the basis of WHO criteria [10]. BMDs of the lumbar vertebrae and the left hips of the subjects were determined using a QDR-2000 dual-energy X-ray absorptiometer (Norland Company, USA) controlled by computers with auto-position fixing, auto-detection and auto-data manipulation. The relative error of repeated detection was 0.5%. BMDs of specific body parts, such as the lumbar vertebrae (L2-L4), the femoral neck, Ward's triangle and the greater trochanter of the femur, were calculated by fan-shaped scanning. The following criteria were considered in this study. BMD is considered normal if T-score is within 1 standard deviation of a normal young adult value. Thus, T-score between 0 and -1 is considered normal; T-score <-1 is considered abnormal. BMD corresponds to low bone mass (osteopoenia) if T-score ranges between -1 and -2.5. This result indicates an increased fracture risk but does not satisfy the criteria of osteoporosis. BMD>2.5 standard deviation from the normal (T score ≤-2.5) value corresponds to osteoporosis.

Determination of serum trace elements

Approximately 4 ml of blood was collected from the ulnar vein of males, who lived in urban areas, in the morning after they underwent overnight fasting. Blood samples were collected into evacuated tubes containing lithium heparin and then stored at -70°C until analysis. The samples were analysed by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent Company, USA) with an octopole-based collision/reaction cell. The samples were subjected to microwave digestion with tetrafluoroethylene in a tank; afterward, 6 ml of concentrated nitric acid was added at room temperature. The solution was allowed to stand overnight in a microwave digestion system and optimised using a microwave digestion program. After the solution was cooled, the acid-digested solution was mixed with catch acid at 150°C for 1 h. The digested solution was subjected to 10-fold dilution by adding ultrapure water to determine zinc and iron contents. The digested solution was not diluted when manganese, selenium, copper, cadmium and lead were determined.

Table 1. Clinical characteristics of the subjects in the three groups

Group	Healthy (N=30)	Osteopenia (N=31)	Osteoporosis (N=30)	*P* value
Age (year)	66.33±10.77	66.56±8.75	63.50±9.74	>0.05[a,b,c]
BMI (kg/m^2)	25.29±2.64	21.22±8.74	22.86±2.30	>0.05[a,b,c]
L2-4 BMD (g/cm^2)	1.068±0.238	1.076±0.143	0.912±0.110	<0.05[b]
L2 BMD (g/cm^2)	1.056±0.203	1.044±0.125	0.903±0.112	<0.05[a,b,c]
L3 BMD (g/cm^2)	1.100±0.203	1.051±0.146	0.852±0.187	<0.05[a,b,c]
L4 BMD (g/cm^2)	1.123±0.287	1.059±0.169	0.884±0.259	<0.05[a,b,c]
Neck BMD (g/cm^2)	0.972±0.094	0.838±0.831	0.660±0.100	<0.05[a, b, c]
Troch BMD (g/cm^2)	0.815±0.150	0.727±0.091	0.604±0.081	<0.05[a,b,c]
Ward's BMD (g/cm^2)	0.771±0.114	0.630±0.128	0.484±0.095	<0.05[a,b,c]

Values are expressed as mean ± SD. *P*<0.05 was considered statistically significant between groups. [a]osteopoenia versus healthy; [b]osteoporosis versus healthy; and [c]osteoporosis versus osteopoenia.

Table 2. Trace element contents of the three groups

Group	Healthy (Group 1) (N=30)	Osteopenia (Group 2) (N=31)	Osteoporosis (Group3) (N=30)	*P* value
Fe (ppm)	454.61±59.98	502.43±40.43	525.03±65.54	<0.05[a]
Mn (ppb)	7.45±10.95	9.86±11.24	5.34±10.45	>0.05
Zn (ppm)	5.23±0.67	5.68±0.67	5.51±0.60	>0.05
Cu (ppb)	833.42±166.84	783.29±107.46	764.40±70.41	>0.05
Se (ppb)	133.97±29.03	144.88±26.81	125.53±22.84	>0.05
Cd (ppb)	0.357±0.509	0.642±0.779	1.239±1.260	<0.05[a]
Pb (ppb)	129.90±121.77	95.60±119.23	116.45±86.51	>0.05

Data are reported as mean ± standard deviation values and compared between groups. *n* total number of subjects. [a]Group 1 vs. 3, *P*<0.05 was considered statistically significant.

Statistical analyses

Data were analysed using SPSS for Windows version 16.0 (SPSS, Chicago, IL, USA). One-way ANOVA was performed to determine statistical significance of differences in variables among groups, and Bonferroni test was conducted as a post-hoc test for multiple comparisons when a significant result was obtained. Correlations between variables were evaluated by Pearson's correlation test. Data were presented as mean ± SD. Statistical significance was set at P<0.05.

Results

Demographic and clinical characteristics of elderly males

A healthy elderly population was selected by excluding subjects with the following: glucocorticoid medication (n=16); renal disease (n=7); cancer (n=8); overt thyroid disease (n=6); and possible rheumatoid arthritis, bone disease other than osteoarthritis, psoriasis or asthma (n=6). Nine subjects who provided insufficient sample volume were also excluded because trace elements could not be measured. Several subjects satisfied two or more criteria, resulting in exclusion of 52 subjects from the initial group of 143 elderly males. A total of 91 elderly males were obtained, and their characteristics are listed in **Table 1**. Age and BMI did not differ among healthy, osteopoenic and osteoporotic groups; by contrast, lumbar and hip BMD significantly differed. The BMDs of the lumbar vertebrae and the femoral neck were lower in osteopoenic and osteoporotic groups than in the healthy group; likewise, BMD of the osteoporotic group was lower than that of the osteopoenic group (P<0.05).

Trace element contents of the three groups

Plasma iron, manganese, zinc, copper, selenium, cadmium and lead contents among the groups are shown in **Table 2**. Plasma manganese, zinc, copper, selenium and lead contents were similar among the groups (*P*>0.05). Iron and cadmium significantly increased in the osteoporosis group compared with those in the healthy group (**Table 2**, *P*<0.05).

Correlation analysis between serum trace element contents and BMD of elderly males

The results of Pearson correlation analysis between serum trace element contents and BMD of elderly males are presented in **Table 3**. Cadmium exhibited a significantly negative correlation with the BMD of the lumbar vertebra (*P*<0.05), particularly L2-4 (r=-0.224), L2 (r=-0.143), L3 (r=-0.237) and L4 (r=-0.242). Manganese contents were positively correlated

Table 3. Pearson correlation analysis between serum elements and BMD (*r*)

	L2-4 BMD r value	L2 BMD r value	L3 BMD r value	L4 BMD r value	Neck BMD r value	Torch BMD r value	Ward's BMD r value
Fe	-0.089	-0.039	-0.068	-0.122	-0.129	-0.076	-0.107
Mn	0.116	0.150	0.127	0.073	0.082	-0.092	-0.135
Zn	-0.097	-0.014	-0.081	-0.145	-0.172	-0.110	-0.174
Cu	-0.013	0.029	-0.021	-0.018	0.019	0.028	0.165
Se	0.011	0.067	0.030	-0.031	-0.038	-0.008	-0.085
Cd	-0.224*	-0.143	-0.237*	-0.242*	-0.051	-0.179	-0.159
Pb	0.051	0.060	0.070	0.027	0.023	0.164	0.190

r Pearson's correlation coefficient; **P*<0.05 was considered statistically significant.

Table 4. Pearson correlation analysis of serum elements (*r*)

	Fe	Mn	Zn	Cu	Se	Cd
Mn	0.378*					
Zn	0.715*	0.422*				
Cu	-0.309*	-0.16	-0.122			
Se	0.477*	0.393*	0.563*	0.016		
Cd	0.155	0.134	0.085	-0.031	0.002	
Pb	-0.185	-0.347*	-0.153	0.245*	-0.177	0.106

**P*<0.05 was considered statistically significant.

with the BMD of the lumbar vertebrae and the proximal femora; by contrast, iron was negatively correlated with BMD. However, this correlation was not statistically significant (*P*>0.05).

Correlation analysis between serum element contents of elderly males

The results of Pearson correlation analysis between serum trace element contents of elderly males are shown in **Table 4**. Serum iron was significantly correlated with manganese, zinc, copper and selenium (*P*<0.05); likewise, serum manganese was significantly correlated with iron, zinc, selenium and lead (*P*<0.05). Serum zinc was also significantly correlated with iron, manganese and selenium (*P*<0.05). Similarly, serum copper was significantly correlated with serum iron and lead (*P*<0.05). Furthermore, serum selenium was significantly correlated with serum iron, zinc and manganese (*P*<0.05). Serum lead was also significantly correlated with serum manganese and copper (*P*<0.05).

Discussion

The causes of osteopenia and osteoporosis are multifactorial, including genetics, endocrine function and exercise and nutritional considerations [11, 12]. Bone formation and metabolism are also modulated by trace elements, such as iron, zinc and copper, in addition to macroelements, such as calcium, phosphorus and magnesium. Trace elements are essential for bone growth and development because these elements interact with bone matrix and affect bone metabolism [6]. These minerals are also implicated in pathology, diagnosis and treatment of osteoporosis [13]. To further clarify the relationship between trace elements and their effect on BMD, we measured serum iron, manganese, zinc, copper, selenium, cadmium and lead contents and analysed their correlation with BMD.

Seven serum trace elements, particularly iron, manganese, zinc, copper, selenium, cadmium and lead, were determined in this study. Iron is involved in the synthesis of collagen and in the conversion of 25-hydroxy vitamin D into an active form [14, 15]. Few studies have shown that iron is essential for proper functioning of osteoblasts and for osteogenic processes [16]. Few studies have also revealed the effect of excess iron on osteoblast dysfunction and metabolic bone disorders, including osteopoenia, osteoporosis and osteomalacia in humans [17, 18]. In our study, iron content was significantly increased in the osteoporosis group compared with that in the healthy group; thus, excess iron in serum may lead to bone mass loss.

Manganese, a component of various enzymes involved in cartilage and bone metabolism [19], is involved in ossification and mucopolysaccharide synthesis in cartilage [20]. Bone disorders caused by manganese deficiency directly result from enzymatic defects in glycosaminoglycan synthesis. However, excess manganese may cause disturbances in the metabolism of other

elements, such as iron, thereby inhibiting haemoglobin formation; this condition causes neurotoxic and osteotoxic effects and disrupts functions of tissues and organs [21, 22]. In our study, serum manganese contents were similar among the groups; thus, our population exhibited normal manganese content.

Zinc, as an activator of numerous metal enzymes, can stimulate activities of bone metabolic enzymes, such as alkaline phosphatase, collagenase and sulfuricoylase; zinc also influences 1,25-0H vitamin D3 and calcitonin concentrations [23, 24]. Zinc can stimulate gene expressions of transcription factors, such as runt-related transcription factor 2, which is related to differentiation forming osteoblastic cells; zinc can inhibit osteoclastic bone resorption by inhibiting osteoclast-like cell formation from bone marrow cells and by stimulating apoptotic cell death of mature osteoclasts [25]. Bone growth retardation is common in various conditions associated with dietary zinc deficiency, suggesting that zinc compounds may be a novel supplement factor in prevention and therapy of osteoporosis [25]. Arikan *et al*. [26] found that zinc is positively correlated with the BMD of the lumbar vertebrae (total T score). Likewise, we observed that zinc levels were similar in all of the groups; this result indicated that dietary zinc was sufficient.

Copper plays an important role in metabolism in the nervous system, haematogenesis, construction of skeleton, connective tissues and cross-linking of elastin and collagen proteins; thus, copper is implicated in bone development and repair [27]. Rodríguez *et al.* [27, 28] concluded that copper stimulates MSC differentiation preferentially toward the osteogenic lineage. Copper deficiency may influence the synthesis and the stability of bone collagen and may induce skeleton development disorders, resulting in osteoporosis. Copper supplementation may be a potential strategy to treat and prevent involutional osteoporosis [29]. In this study, copper concentrations were similar among healthy, osteopoenic and osteoporotic groups.

A group of paediatric patients with low BMD present low selenium status caused by low-selenium formula diets [30]; this condition has also been observed in residents of Tibet where selenium content in soils is low [31]. These findings are possibly related to the functions of selenoproteins. Many, if not all, selenoproteins are antioxidant enzymes necessary to maintain cell redox balance, which is essential for the regulation of inflammation and bone cell proliferation/differentiation [32]. Osteoclasts are activated by inflammatory cytokines released by osteoblasts at low levels [33]. Thus, selenium can alleviate NF-κB-dependent regulation of inflammatory responses; this result suggests that selenium may mediate osteoblast-osteoclast crosstalk [34]. In this study, selenium was not related to BMD in normal and osteoporotic groups.

Bone is one of the target organs of cadmium toxicity [35]. Cadmium exposure can induce bone loss, lead to osteoporosis and increase the risk of bone fractures in humans and experimental animals [36-42]. In our study, cadmium concentrations were higher in the osteoporotic group than in the healthy group; cadmium concentrations also differed between normal BMD and osteoporosis. Cadmium exposure exhibited a significantly inverse association with BMD. Our results also showed a significant inverse association between cadmium exposure and BMD; cadmium levels were higher in the osteoporotic group than in the healthy group. Moreover, cadmium levels differed between normal BMD and osteoporosis.

Cadmium and lead levels of individuals living in polluted areas are significantly higher than those living in controlled areas; this result suggested that these elements may affect bones and interactively affect BMD [43]. Lead may also interact with other factors in the course of postmenopausal osteoporosis; lead further inhibits vitamin D activation, dietary calcium uptake and several regulatory aspects of cell function, thereby aggravating the course of this disease [44]. Animal studies have demonstrated that increased lead exposure is associated with decreased bone density [45-47] and bone strength [48]. Moreover, in vitro studies have revealed that lead exposure inhibits the function of chondrocytes and osteoblasts in bone development [48, 49]. In another study, a significant inverse association has been observed between lead exposure and BMD among white subjects [50]. In our study, BMD was similar among healthy, osteopoenic and osteoporosis groups; thus, these factors may have not been influenced by lead.

This study demonstrated that iron and cadmium were significantly increased in the osteoporosis group compared with those in the healthy group. Our results further revealed that serum iron and cadmium contents may directly and mutually influence BMD; these trace elements may affect the pathogenesis of osteoporosis. Serum cadmium content was also significantly and negatively correlated with BMD; this result suggested that cadmium could be involved in the development of osteoporosis. Furthermore, our results demonstrated that these trace elements directly and correlatively influenced BMD; indeed, these elements may affect the pathogenesis of osteoporosis. However, the relationship of these trace elements with BMD and osteoporosis remains unclear. As such, future studies should be conducted to evaluate serum and bone levels of these trace elements to determine the relationship of these trace elements with osteoporosis.

Disclosure of conflict of interest

None.

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