

A Comparison of Serum Lead Status among Elderly Osteopaenic Patients, Elderly Osteoporotic Patients and Healthy Controls

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ABSTRACT

Objective: It is proposed that in some conditions such as pregnancy and osteoporosis where the bone turnover rate is high, there is mobilization of various minerals including lead (Pb) from bone to blood. This study aimed to determine if there were any differences in serum Pb levels among elderly osteopaenic patients, elderly osteoporotic persons and healthy controls.

Methods: Fifty-four elderly persons (26 men and 28 women) from the Amirkola Health and Ageing Project, Iran, were included in this study. The diagnosis of osteopaenia and osteoporosis was based on spine and femur bone mineral density (BMD) measurements. After blood sampling, serum Pb levels were analysed by the method of atomic absorption spectrophotometry.

Results: According to the BMD measurements, 19 persons had normal BMD, while 16 had osteopaenia and 19 suffered from osteoporosis. The differences in body mass index in these three groups were statistically significant ($p < 0.001$). The patients with osteoporosis had the highest levels of alkaline phosphatase and the highest rate of bone turnover. The mean \pm standard deviation of the serum Pb levels in these groups were 236.8 ± 98.0 , 270.0 ± 81.5 and 258.3 ± 57.5 $\mu\text{g/L}$, respectively, and the differences were not statistically significant ($p = 0.467$).

Conclusion: No statistically significant differences in serum Pb levels were observed in healthy controls compared with osteopaenic persons and osteoporotic persons. This suggests that mobilization of Pb from bone to blood in this population of elderly osteopaenic patients and elderly osteoporotic patients was similar to that in the healthy controls.

Keywords: Lead, mobilization, osteopaenia, osteoporosis

Comparación del estado de plomo sérico entre los pacientes osteopélicos mayores de edad, los pacientes osteoporóticos mayores de edad, y los controles sanos

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RESUMEN

Objetivo: Se postula que en algunas condiciones como el embarazo y la osteoporosis donde el índice de recambio óseo es alto, hay movilización de varios minerales – incluyendo plomo (Pb) – de los huesos a la sangre. Este estudio tuvo como objetivo determinar si hubo diferencias en los niveles de plomo sérico entre los pacientes osteopélicos mayores de edad, los pacientes osteoporóticos mayores de edad, y los controles sanos.

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Métodos: Se incluyeron en este estudio 54 personas de edad avanzada (26 hombres y 28 mujeres) del Proyecto Amirkola de Salud y Envejecimiento, Irán. La diagnosis de la osteopenia y la osteoporosis se basó en mediciones de la densidad mineral ósea (DMO) de la espina dorsal y del fémur (DMO). Después del muestreo de sangre, los niveles séricos de Pb fueron analizados por el método de espectrofotometría de absorción atómica.

Resultados: Según las mediciones de la DMO, 19 personas tenían DMO normal, mientras que 16 tenían osteopenia y 19 padecían osteoporosis. Las diferencias en el índice de masa corporal en estos tres grupos fueron estadísticamente significativas ($p < 0.001$). Los pacientes con osteoporosis tenían los niveles más altos de fosfatasa alcalina y el índice más alto de recambio óseo. La media \pm desviación estándar de los niveles séricos de Pb en estos grupos fue de 236.8 ± 98.0 , 270.0 ± 81.5 y 258.3 ± 57.5 $\mu\text{g/L}$, respectivamente, y las diferencias no fueron estadísticamente significativas ($p = 0.467$).

Conclusión: No se observaron diferencias estadísticamente significativas en los niveles séricos de Pb en los controles sanos en comparación con las personas osteopénicas y las osteoporóticas. Esto sugiere que la movilización de Pb del hueso a la sangre en esta población de pacientes osteopénicos mayores de edad y pacientes osteoporóticos mayores de edad, era similar a la encontrada en los controles sanos.

Palabras clave: Plomo, movilización, osteopenia, osteoporosis

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INTRODUCTION

Lead (Pb) is a toxic metal that is able to affect human health by various methods. Exogenous Pb is one of the most important methods of Pb contamination (1). Researchers have shown that some humans are also at risk of endogenous exposure to Pb previously accumulated in bone and skeleton (2, 3). The human skeleton is in a state of dynamic turnover; there is active mineral metabolism in it, and many factors affect this procedure (4–6). It is proposed that during life, Pb and calcium are deposited in the skeleton and may act similarly. Calcium and Pb compete for transport and binding sites; a study proposed that in conditions where the liberation of calcium occurred from bone, Pb was also liberated (7). There was evidence that where an increase in the rate of bone demineralization was observed (eg in pregnancy, lactation, menopause and also in osteopathic diseases such as osteoporosis), mobilization of Pb from bone to blood was probable (8). Silbergeld *et al* reported that after mobilization of Pb from bone to blood, released Pb contributed to inhibiting the activation of vitamin D and also the uptake of dietary calcium (9). Tsaih *et al* observed that bone resorption could influence the release of bone Pb stores into the blood stream (10).

It seems that during the osteopathic disorders, release of Pb from bone to blood can aggravate the disease. Released Pb is able to prevent the activation of vitamin

D, and the result of vitamin D inactivation is calcium deficiency (7, 8). When the bone turnover is high, Pb is released from bone and ultimately contributes to more weakness of bone. When this process occurs in elderly persons, they will become more susceptible to falls and fractures.

There are reports regarding the status of blood Pb in persons with a higher exposure to environmental Pb (11, 12), but in elderly persons and persons with a lower exposure to environmental Pb, where the contribution of endogenous Pb to blood may be more relevant, we did not find any reported data. Therefore, we aimed to determine the status of blood Pb in a population with a lower exposure to environmental Pb. To this end, we compared the level of Pb in the blood of elderly osteopaenic patients and elderly osteoporotic patients who have a high rate of bone turnover with that of healthy controls.

SUBJECTS AND METHODS

Study population

The study population comprised elderly persons from the Amirkola Health and Ageing Project (13). The project was a population-based cross-sectional study in which people aged ≥ 60 years were recruited for the health study. In this present study, we determined the status of osteopaenia and osteoporosis in the elderly persons by

measurements of bone mineral density (BMD). Among the patients who had osteopathy, we separated randomly those who did not receive any bone-affecting drugs from age-matched healthy controls. Fifty-four elderly persons were considered for this study and had their BMD measured. The diagnosis of osteopaenia and osteoporosis was based on lumbar spine and femoral neck BMD measurements using dual energy X-ray absorptiometry with the Lexxos densitometer according to the following criteria of the World Health Organization:

- Osteoporosis: BMD that lies 2.5 standard deviations (SD) or more below the average value for young healthy adults, *ie* a T-score of < -2.5 SD
- Osteopaenia: a T-score between -1.0 and -2.5 SD
- Healthy: a T-score greater than -1.0 (14).

Fasting blood samples were obtained from all participants during the primary examinations, and serum aliquots were stored at -80° C until analysis. The study was approved by the ethics committee of Babol University of Medical Sciences, Iran.

Biochemistry analysis

After collecting the blood and separating the serum, some routine biochemistry tests (such as calcium, phosphorous, parathyroid hormone (PTH), alkaline phosphatase (ALP) and vitamin D status) were analysed by commercially available kits. Calcium, phosphorous and ALP were assessed by spectrophotometer (CamSpec-m501, China) according to the cresolphetalein, phosphotungestate and P-Nitrophenyl phosphate methods (Pars Azmoon kit, Tehran, Iran), respectively. In addition, the levels of PTH and vitamin D were determined by an ELISA reader (Rayto-RT-2100, China) with intact parathyroid hormone and 25-hydroxy vitamin D (IDS Company, England) kits.

Determination of serum lead levels by atomic absorption spectrophotometry

A PG990 atomic absorption spectrophotometry (AAS) machine equipped with deuterium background correction and Pb hollow-cathode lamp as the radiation source was used for analysis of the serum Pb level. Argon was used as purge gas for the graphite furnace. The operating parameters were set as recommended by the manufacturer (wavelength 283.3 nm, slit width 0.7 nm and current 10 mA). The instrument settings and furnace programme for Pb analysis are presented in Table 1.

Table 1: Settings of atomic absorption spectrophotometry, and furnace programme for serum lead analysis

Step	Temperature (°C)	Ramp (second)	Hold (second)	Argon flow (ml/minute)
Drying	70	10	10	300
Drying	110	10	10	300
Pyrolysis	450	10	15	300
Atomization	1800	0	3	0
Clean-up	1900	1	2	300

Stock standard solutions of Pb at a concentration of 1000 mg/L (parts per million) in a solution containing 0.5 mol HNO₃ (ultra-pure) were provided from Merck. De-ionized water was used to prepare serial solutions for preparing various working standard solutions, *ie* 0, 0.312, 0.625, 1.25, 2.5, 5 and 10 µg/L (parts per billion). A volume of 20 µL of the final solution was injected into the graphite tube.

The coefficient of variation (CV) of the method was calculated by dividing the SD of the determined Pb by the mean, multiplied by 100. For determination of the method repeatability, we analysed the levels of Pb in six samples three times and calculated the CV.

Statistical analysis

The data were analysed by the SPSS for Windows version 17.0. The results were expressed as mean value \pm SD, and a *p*-value of < 0.05 was considered statistically significant. The mean values obtained in the three groups (osteoporosis, osteopaenia and healthy controls) were compared by the one-way ANOVA test, scheffe method. For analysis of the correlation between various parameters, Pearson correlation analysis was used.

RESULTS

According to the BMD measurements, 19 persons had normal BMD, 16 had osteopaenia and 19 had osteoporosis. The mean \pm SD of their ages were 65.6 ± 5.6 , 65.4 ± 4.8 and 69.4 ± 6.3 years, respectively. The mean \pm SD of their body mass index was 30.3 ± 3.9 , 26.7 ± 3.5 and 24.1 ± 4.7 kg/m², respectively, and the differences were statistically significant among the groups ($p < 0.001$). Table 2 presents the demographic and BMD characteristics of the study participants.

The CV of the AAS method for measuring the levels of Pb in the samples was less than 8.1%. This emphasizes the repeatability of the method.

Table 3 shows that there were no statistical differences in the participants' levels of calcium, phosphorus, PTH and vitamin D (the corresponding *p*-values were all

> 0.05), but the levels of ALP in the three groups were statistically different and the patients with osteoporosis had the highest levels of ALP ($p = 0.008$).

Figure 1 presents the levels of Pb in elderly patients with osteoporosis, elderly patients with osteopaenia,

Table 2: Demographic and bone mineral density results of the study participants

Variables	Healthy controls (n = 19)	Osteopaenia (n = 16)	Osteoporosis (n = 19)
Age (years)	65.6 ± 5.6	65.4 ± 4.8	69.4 ± 6.3
Height (cm)	160.3 ± 8.8	158 ± 6.8	153.2 ± 8
Weight (kg)	77.5 ± 10.5	66.5 ± 9.1	56.3 ± 10.3
Bone mineral density of spine (g/cm ²)	1.11 ± 0.13	0.86 ± 0.03	0.62 ± 0.09
Bone mineral density of femur (g/cm ²)	1.05 ± 0.1	0.85 ± 0.05	0.65 ± 0.08
Spine T score	0.3 ± 0.91	-1.6 ± 0.3	-3.7 ± 0.9
Femur T score	0.1 ± 0.7	-1.4 ± 0.4	-2.9 ± 0.6
Spine Z score	1.3 ± 0.8	-0.63 ± 0.43	-2.17 ± 0.79
Femur Z score	0.9 ± 0.5	-0.66 ± 0.4	-1.6 ± 0.9
Body mass index (kg/m ²)	30.3 ± 3.9	26.7 ± 3.5	24.1 ± 4.7

Table 3: Comparison of various biochemical variables of the study participants

Variables	Healthy controls	Osteopaenia	Osteoporosis	p-value
Lead (µg/L)	236.8 ± 98	270 ± 81.5	258.3 ± 57.5	0.467
Calcium (mEq/L)	9.2 ± 0.4	9.3 ± 0.5	9.1 ± 0.3	0.464
Phosphorus (mEq/L)	3.7 ± 0.4	3.7 ± 0.6	3.9 ± 0.5	0.303
Parathyroid hormone (ng)	43.7 ± 16.5	56.5 ± 45.0	52.6 ± 28.5	0.609
Alkaline phosphatase (u/L)	208.4 ± 58.6	213.1 ± 59.6	280.2 ± 97.9	0.008
Vitamin D (nmol/L)	37.8 ± 38.5	17.6 ± 7.2	30.5 ± 24	0.184
Body mass index (kg/m ²)	30.3 ± 4	26.7 ± 3.5	24.1 ± 5	0.000

and healthy controls. Although we could see a gradual increase in the levels of Pb from healthy controls towards those with bone disorders in the crude data, the statistical analysis did not show any statistically significant differences ($p = 0.467$). Figure 2 shows the relationship between levels of serum Pb and BMD in the study population.

DISCUSSION

Research has shown that Pb is an element that accumulates in bone (4). From childhood to adolescence, accumulated Pb is deposited in bone (15). When there is an increase in bone turnover (such as in pregnancy and menopause), it seems that Pb is mobilized from bone to blood. A study by Ronis *et al* showed that Pb could affect the action of osteoblast and also osteoclast in a direct or indirect manner (16).

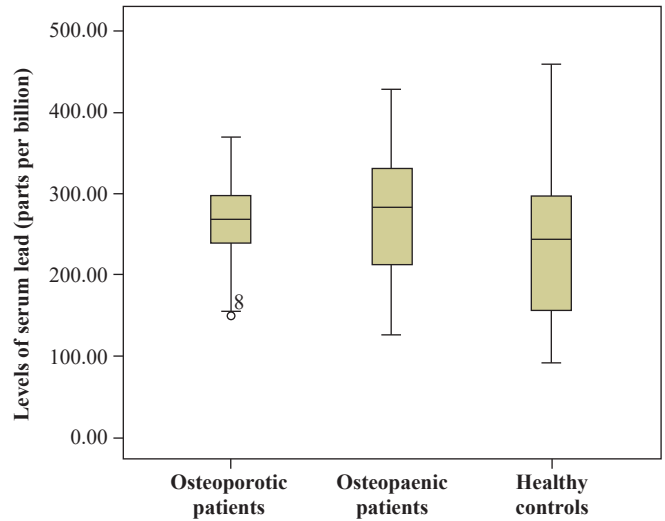


Fig. 1: Box plots of levels of serum lead (parts per billion) in patients with osteoporosis (n = 19), patients with osteopaenia (n = 19) and healthy controls (n = 16).

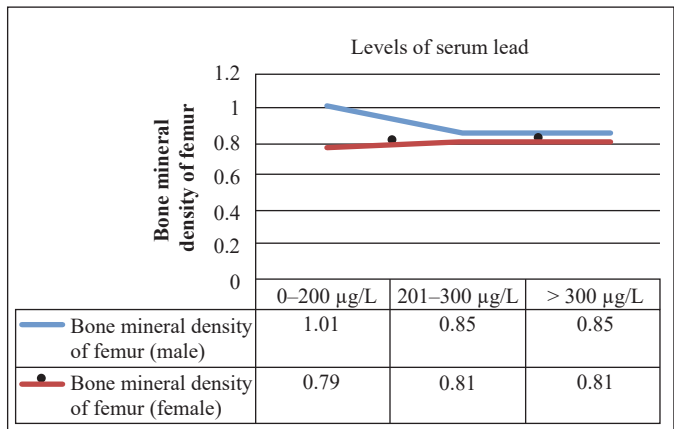
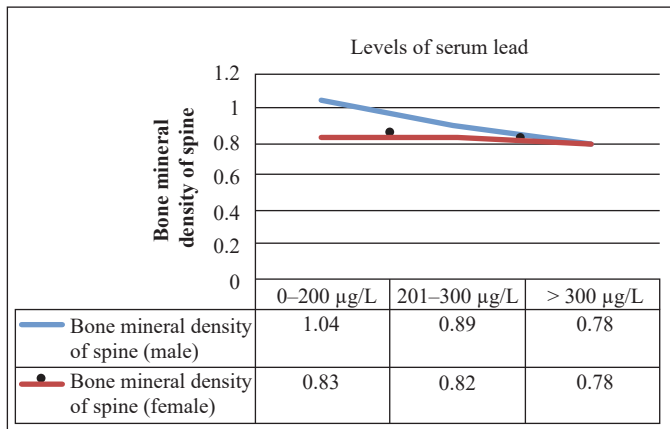


Fig. 2: Relationship between levels of serum lead and bone mineral density in the study population, according to gender and the exact amounts of lead based on the spinal and femoral bone mineral density.

Current exposure to Pb and mobilization from bone to blood are reflected in an increased blood lead level (9, 17). We aimed to determine whether there was mobilization of Pb from the skeleton to the blood in elderly persons with bone disorders. Higher levels of serum Pb can contribute to bone fracture risk.

We observed that the patients with bone disorders had lower BMD and body mass index and their levels of serum calcium, phosphorus, PTH and vitamin D compared with those of the healthy group were not statistically significant. It was expected that the levels of ALP in the three groups would be statistically different. The patients with osteoporosis had the highest levels of ALP, and this means a high rate of bone turnover. In persons with osteopaenia and persons with osteoporosis, the serum levels of Pb were greater than those in the healthy controls in the crude data, but by statistical analysis, no significant difference was observed. It seems that persons with higher levels of BMD had lower concentrations of Pb in their blood. This pattern is more powerful in the males than the females. We think that there was a gradual move of bone Pb stores to the blood in those with bone disorders, but due to the small sample size, the differences in comparison with healthy controls were not statistically significant. Overall, this suggests that mobilization of Pb from bone to blood in our elderly osteopaenic patients and elderly osteoporotic patients was similar to that in the healthy controls.

Reported studies regarding the status of levels of blood Pb in osteopaenic patients and osteoporotic patients are controversial (18). Silbergeld *et al* analysed the status of blood Pb in women and concluded that when mobilization of Pb from bone to blood had occurred, released Pb contributed to worsening the course of the postmenopausal osteoporosis (9). In another report, Tsaih *et al* evaluated the effect of bone resorption on the release of Pb from bone in middle-aged and elderly men and concluded that bone resorption could influence the release of bone Pb stores into the blood (10). Hu *et al* measured the levels of blood Pb and Pb in the tibia and patella bones in middle-aged men who had no occupational exposures to Pb and reported that bone Pb levels were the major source of blood Pb in these persons (19). In another study, on 1225 women, researchers evaluated the influence rate of bone turnover on the amount of Pb release from bone to blood. They found a significantly positive correlation between markers of bone turnover (*ie* N-telopeptide cross-linked collagen type I (NTx), bone-specific alkaline phosphates (BALP) and osteocalcin (OC)) and the levels of Pb in the blood.

They concluded that where the rate of bone turnover was high, the stored Pb from bone would be released into the blood (3).

In addition to the above-mentioned research, there are other reports on the Pb status of persons who have been exposed to Pb. For example, in a study involving 155 male workers who had been exposed to Pb, the authors observed that persons with occupational exposure to Pb may develop some chronic diseases such as osteoporosis and renal dysfunction (20). In another study, researchers found that there were no associations between BMD and Pb levels, but observed a strong association between cadmium exposure and BMD (21). Campbell and Auinger reported a significant inverse association between exposure to Pb and the amounts of BMD (11). Khalil *et al* investigated the association between levels of blood Pb and fractures. They observed that women with higher levels of blood Pb had an increased risk of fracture and concluded that a higher level of blood Pb was one of the risk factors of falls and fractures (22).

The present study has some limitations. Lead is excreted through kidneys, but we did not analyse the status of the renal function of the participants. In addition, one of the major routes that Pb enters the body is food, but we did not measure the amount of dietary intake of Pb.

CONCLUSION

The results of the present study suggest that the status of blood Pb of the elderly osteopaenic patients and elderly osteoporotic patients was similar to that of the healthy controls. A more comprehensive study with a larger sample size is needed to reach a conclusive statement regarding this issue.

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