Determination of Blood Calcium and Lead Concentrations in Osteoporotic and Osteopenic Patients in Pakistan

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ABSTRACT: Osteoporosis is the leading cause of deformity and bones fracture all over the world and has some relationship with the blood concentrations of calcium and lead. Therefore, in the current study, the blood samples of 58 control and 56 clinically diagnosed osteoporotic and osteopenic patients were taken from different hospitals in Pakistan and analyzed for calcium and lead concentrations using atomic absorption spectrometry. In female control samples, the mean calcium value was found to be 98.53 ± 4.81 μg/mL, and in male control samples, the mean blood calcium level was found to be 121.33 ± 7.27 μg/mL. In female control samples, the mean lead value was found to be 0.133 ± 0.005 μg/mL, and in male control samples, the mean lead level was found to be 0.183 ± 0.008 μg/mL. All the male and female control samples showed a mean value of calcium of 115.63 ± 5.2 μg/mL and a mean value of lead of 0.153 ± 0.007 μg/mL. In osteoporotic female patients, the decline in the mean calcium value was found to be 34.93 ± 1.9 μg/mL, and in male patients, the decrease in the mean calcium level was found to be 47.73 ± 2.5 μg/mL. The increase in the mean value of lead in osteoporotic females was 4.13 ± 0.22 μg/mL, whereas in male patients, the increase in the mean lead value was 0.95 ± 0.07 μg/mL. All the male and female patients showed a decrease in the mean value of calcium of 41.43 ± 2.2 μg/mL and an increase in the mean value of Pb of 3.63 ± 0.16 μg/mL.

INTRODUCTION

Osteoporosis, a common bone disorder, is characterized by reduced bone density and altered bone microarchitecture. Osteoporosis is prevalent in postmenopausal females and elderly males. Women are at greater risk of developing osteoporosis than men. In 2003, the World Health Organization (WHO) reported that 70 million people had osteoporosis worldwide. To date, this number has substantially increased many times; however, no clear figures about osteoporotic cases per year are yet available. Some 44 million people in the United States are at risk of osteoporosis. Osteoporosis is a major health concern in the world including Pakistan. A great number of people in Pakistan are affected by osteoporosis. In 2013, a study reported that 40 million people are affected by osteopenia, while almost 10 million people are suffering from osteoporosis in Pakistan. In osteoporotic patients, the bone mineral density (BMD) decreases because of demineralization of bones. Thus, bones become weak and fragile, resulting in fracture, which occurs at almost any site but most commonly on the hip, the vertebral spine, and the wrist.

Bone is a living growing tissue and is made mostly of collagen, a protein that provides a soft framework, and calcium phosphate, a mineral that adds strength and hardens to the framework of bones. Bone cells called osteoblasts build bone, while other bone cells called osteoclasts break down bone. Throughout the lifespan, the bones are constantly being broken down and built up in a process known as remodeling. Bones need nutrition like any other tissue. Analysis of human bones can reveal dozens of essential metals along with trace quantities of toxic elements.

Calcium (Ca) is an important mineral that is required for building and maintaining bones and teeth. The body gets the required Ca in two ways. One way is by eating foods or supplements that contain Ca. For the other way, the body

Received: August 22, 2021
Accepted: September 28, 2021
Published: October 12, 2021
gets Ca by pulling it from bones. Whenever the concentration of Ca in the blood of human beings becomes low due to not getting enough Ca from food, then Ca is released from the bones and becomes a part of blood; thus, osteoporotic conditions are observed.\(^{12,13}\) So it is inferred that the level of Ca in blood has some relationship with osteoporosis.

Lead exposure may be a risk factor in the development of osteoporosis. Lead is accumulated in bones and replaces Ca from bones due to having the same charge density as Ca.\(^{14}\) Animal studies report that increased lead exposure is associated with a decrease in bone density and bone strength.\(^{15–18}\) Additional in vitro studies report that lead may affect the growth plate chondrocytes in cell culture and cause an inhibitory effect on the process of endochondral bone formation.\(^{19}\) Another in vitro study suggests that lead toxicity can affect osteoblast function that may contribute to the skeletal abnormalities.\(^{20}\)

The literature showed that there was a relationship between the levels of blood Ca and Pb and osteoporosis,\(^ {19–21}\) but no such study was conducted in Pakistan. Therefore, in the current study, the blood calcium and lead concentrations of both normal and osteoporotic and osteopenic patients of different regions of Pakistan were determined using atomic absorption spectrometry and compared. The concentrations of Ca and Pb in blood samples can serve as a useful indicator of the bone disease osteoporosis.

**RESULTS AND DISCUSSION**

The blood samples of control and osteoporotic+ osteopenic patients were analyzed for Ca and Pb by using a flame atomic absorption spectrometer. The 58 controls who volunteered including 33 females and 25 males (with age ranging from 28 to 65 years) along with 56 patients including 39 osteoporotic females and 17 osteopenic males (with age ranging from 45 to 83 years) donated blood for the said study. All the samples were collected from different hospitals in Pakistan with the proper consent of the volunteers. The optimized instrumental conditions for the analysis of Ca and Pb by flame atomic absorption spectrometry are given in Table 1.

### Table 1. Optimized Instrumental Parameters for Calcium and Lead Determination

| parameters                      | calcium | lead |
|---------------------------------|---------|------|
| lamp current (mA)               | 7.5     | 7.5  |
| wavelength (nm)                 | 422.7   | 283.3|
| slit (nm)                       | 1.3     | 1.3  |
| burner head                     | standard| standard|
| burner height (mm)              | 7.5     | 7.5  |
| flame                           | air–acetylene | air–acetylene |
| oxidant gas pressure (kPa)      | 160     | 160  |
| fuel gas flow rate (L/min)      | 2.2     | 2.0  |

The mean blood Pb concentration (0.153 \(\mu\)g/mL) of controls in our study is higher than the permissible limit of the WHO, i.e., 0.1 \(\mu\)g/mL. If we compare the mean blood Pb level of controls of our study, the value was found to be globally lower than that of the healthy population of India (0.212 \(\mu\)g/mL). On the other hand, the mean blood Pb concentration of controls (0.153 \(\mu\)g/mL) in our study is comparable to the value for Belgium (0.14 \(\mu\)g/mL) and is higher than that of the healthy population of USA (0.092 \(\mu\)g/mL), Sweden (0.025 \(\mu\)g/mL), Japan (0.049 \(\mu\)g/mL), and UK (0.122 \(\mu\)g/mL), as shown in Table 3. The high lead concentration in populations of Pakistan, India, and Belgium may be due to occupational lead exposure, industrial emission, ingesting lead dust or paint chips, and eating contaminated food or drinking contaminated water. The blood lead level in populations of USA, UK, Sweden, and Japan has decreased drastically since lead was eliminated from house paints, gasoline, water pipes, and other household products and because of the close monitoring of lead in the industry.\(^ {23–24}\)

Concentrations of Calcium and Lead in Control Samples. The determined concentrations of Ca and Pb in control samples with mean values and standard deviations are depicted in Table 2.

From Table 2, it is clear that the blood calcium concentration in control subjects comes out to be 98.53 ± 4.81 \(\mu\)g/mL, while in male subjects, it is 121.33 ± 7.27 \(\mu\)g/mL. The mean concentration of Pb in control samples of both female and male subjects of Islamabad and Lahore was found to be 0.133 ± 0.005 \(\mu\)g/mL. The mean blood Pb concentration of male control samples of both Islamabad and Lahore was found to be 0.183 ± 0.008 \(\mu\)g/mL. Therefore, the average normal concentration of Pb in female and male subjects of both areas comes out to be 0.153 ± 0.007 \(\mu\)g/mL. The data taken in the questionnaire indicate that normal subjects did not have any occupational exposure of Pb (like working in the industry) or habitual exposure (like smoking or use of alcohol, etc.).

For a global comparison, the reported blood Pb concentrations of control samples from different countries are also summarized in Table 3.

**Table 2. Concentrations of Lead and Calcium in Control Samples**

| number of samples | age range | sex | Ca range, mean ± SD (\(\mu\)g/mL) | Pb range, mean ± SD (\(\mu\)g/mL) |
|-------------------|-----------|-----|---------------------------------|---------------------------------|
| 33                | 28–57     | F   | 78.2–113, 98.53 ± 4.81          | 0.09–0.19, 0.133 ± 0.005         |
| 25                | 30–65     | M   | 125–147, 121.33 ± 7.27          | 0.14–0.23, 0.183 ± 0.008         |
| total: 58         | 28–65     | F + M | 78.2–147, 115.63 ± 5.2         | 0.09–0.23, 0.153 ± 0.007         |

**Table 3. Blood Pb Concentration (\(\mu\)g/mL) of Healthy Populations of Different Global Countries**

| sr. no. | countries | Pb (\(\mu\)g/mL) | ref |
|---------|-----------|-----------------|-----|
| 1       | Sweden    | 0.025           | 22  |
| 2       | India     | 0.212           | 22  |
| 3       | Belgium   | 0.140           | 23  |
| 4       | Japan     | 0.049           | 23  |
| 5       | USA       | 0.092           | 23  |
| 6       | UK        | 0.122           | 24  |
| 7       | present study | 0.153       |     |

The mean blood Pb concentration (0.153 \(\mu\)g/mL) of female control samples of Islamabad and Lahore was found to be 0.133 ± 0.005 \(\mu\)g/mL. The mean blood Pb concentration of male control samples of both Islamabad and Lahore was found to be 0.183 ± 0.008 \(\mu\)g/mL. Therefore, the average normal concentration of Pb in female and male subjects of both areas comes out to be 0.153 ± 0.007 \(\mu\)g/mL. The data taken in the questionnaire indicate that normal subjects did not have any occupational exposure of Pb (like working in the industry) or habitual exposure (like smoking or use of alcohol, etc.).

Concentration of Calcium and Lead in Osteoporotic and Osteopenic Patients. In the next experiment, the blood samples of 56 clinically diagnosed osteoporotic and osteopenic patients (female: 39 and male: 17) were analyzed for Ca and Pb levels, and the results are shown in Table 4 along with means and standard deviations. The mean Ca level determined in osteoporotic and osteopenic patients was found to be 34.93...
Comparison of the concentration of blood Ca of osteoporotic and osteopenic patients with that of normal subjects. Studies showed that there was a significant decrease in intestinal Ca absorption with age. Another possible reason for the decreased calcium level and osteoporosis at older age may be the absence of estrogen especially in females due to which minerals may be lost from bones as depicted in the literature. Estrogen is a sex hormone that is essential to female bone health because it promotes the activity of osteoblasts, the cells that produce bone. When estrogen levels drop during menopause at older age, the osteoblasts are not able to effectively produce bones. Similarly, in aging men, estrogen is a sex steroid that is responsible for the regulation of the bone resorption phenomenon more than testosterone. With age, the substantial decrease in these hormones leads to osteoporosis. Socioeconomic status is also a significant and strong predictor of many health problems. The individuals included in our study belong to middle class and poor family backgrounds and thus have osteoporosis. A study shown by Navarro et al. in 2013 indicates that poor socioeconomic status was associated with 25-OH D insufficiency, higher values of the parathyroid hormone (PTH) and lower values of BMD at the lumbar spine, and a higher prevalence of fragility fractures.

On the other hand, if we compare the concentration of Pb in control and osteoporotic and osteopenic groups, we notice that a high level of Pb was found in osteoporotic patients for both males and females, and the results are shown in Figure 2.

The decrease in blood Ca level for both males and females (diseased) may be highly attributed to the low consumption of milk and dairy products in the diet as recorded in the questionnaire. The questionnaire data showed that out of 56 clinically diagnosed osteoporotic and osteopenic patients, only 5 individuals took milk at the age of 10 years, while the remaining 51 took milk at the age of 5 years or less than that. The absence of milk in the diet or the very low intake could be responsible for low Ca levels that contribute to the development of osteoporosis and osteopenia. Many published studies showed that low Ca intake appears to be associated with low bone mass, rapid bone loss, and high fracture rates. The second possible reason could be the decreased intestinal Ca absorption in older osteoporotic patients. Studies showed that there was a significant decrease in intestinal Ca absorption with age. Another possible reason for the decreased calcium level and osteoporosis at older age may be the absence of estrogen especially in females due to which minerals may be lost from bones as depicted in the literature. Estrogen is a sex hormone that is essential to female bone health because it promotes the activity of osteoblasts, the cells that produce bone. When estrogen levels drop during menopause at older age, the osteoblasts are not able to effectively produce bones. Similarly, in aging men, estrogen is a sex steroid that is responsible for the regulation of the bone resorption phenomenon more than testosterone. With age, the substantial decrease in these hormones leads to osteoporosis.

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CONCLUSIONS

Osteoporosis and osteopenia are widespread bone diseases all over the world including Pakistan. In this study, the concentration of Ca and Pb in the whole blood of 58 normal individuals and 56 osteoporotic + osteopenic patients of different regions of Pakistan was taken. The samples were acid digested and analyzed by using atomic absorption spectrometry to check the imbalance of these metals ions. All diseased patients including females and males of different age groups have decreased Ca and increased Pb levels in their blood as compared to control samples. This is because Pb and Ca compete for binding and transport sites, leading to the uptake of Pb in bones. During osteoporosis and osteopenia, Pb is mobilized from bones to blood. The current study shows that there is a relationship between the levels of blood Ca and Pb and osteoporosis. Therefore, the determination of these metals may be beneficial parameters for the clinical evaluation and diagnosis of osteoporosis.

EXPERIMENTAL SECTION

Instrumentation. All the quantification was carried out using a Hitachi model Z-2000 Polarized Zeeman Atomic Absorption Spectrometer coupled with a software-based data handling facility and a printer.

Reagents. Glassware was cleaned by soaking in 0.1 mol dm$^{-3}$ HNO$_3$ overnight followed by repeated rinsing with distilled water. The concentrated acids HClO$_4$ and HNO$_3$ (Merck, Germany) used were of analytical grade. Standard stock solutions (1000 μg/mL) of Ca and Pb were used in preparing subsequent calibration curve after some serial dilutions. For the preparation of calibration curve, fresh working standards were made by the appropriate dilution of a stock solution of 1000 μg/mL in distilled deionized water immediately before use. In Ca analysis, 1000 μg/mL Sr was used as an ionization suppressor as Ca is easily enhanced in air–acetylene flame.

Procedure. The whole blood samples of 58 normal and 56 osteoporotic + osteopenic patients including females and males were collected from different hospitals of Islamabad and Lahore cities in Pakistan. In this study, the femoral neck T-score was considered as a criterion for the selection of osteoporotic patients. The T-score is a comparison of the patient’s bone density with that of healthy, young individuals of the same sex. A negative T-score of −2.5 or less at the femoral neck defines osteoporosis. Controls had a normal femoral neck density (T-score ≥ 1), whereas osteoporotic patients had a T-score < 1. However, in this study, all the 17 males with osteopenia had a T-score ≥ 1. A questionnaire was designed including the history and a brief biodata of patients so that the effect of age, sex, socioeconomic status, and dietary habits was evaluated regarding osteoporosis. The blood samples were collected in blood collecting vials (EDTA K$_3$; Nanchang Ganda Medical Devices Co, Ltd.) containing EDTA as an anticoagulating agent. EDTA has been recommended as the anticoagulant of choice for hematological testing because it allows the best preservation of cellular components and morphology of blood cells. The whole blood samples (about 4 mL) were collected by direct venous puncture with the help of a new sterilized syringe under the supervision of the medical staff of the respective hospital. The puncture sites were cleaned before sampling by using alcohol to reduce the chances of contamination. The blood was shifted to vials and shaken thoroughly so that it was mixed properly with the anti-coagulating agent to avoid clotting. Digestion of the samples was done within 24 to 48 h; however, if sometimes it is not possible to digest all the samples immediately, then the samples were stored in a refrigerator and the temperature was maintained at 4 °C.

For the digestion of samples, about 0.5 mL of blood samples was taken in triplicate in 100 mL digestion flasks fitted with a 30 cm long air condenser. Then, 0.5 mL of concentrated HNO$_3$ was added to the sample. The contents were heated at 80 °C for 30 min. After cooling, 1.5 mL of concentrated HClO$_4$ (70%) was added and heated again at 250 °C with occasional shaking till white fumes evolved. The clear solution obtained was cooled and transferred into a 10 mL measuring flask, and the volume was made up with deionized water for subsequent measurements of metals. A blank was prepared under similar conditions. Calibration standard solutions were prepared for each element separately from the stock solution in 0.02 N HNO$_3$. Blood samples were diluted accordingly with 0.02 N HNO$_3$. The absorbance values of a specific metal were measured by aspirating the solutions into air–acetylene flame employing the optimized conditions given in Table 1. A minimum of three absorbance values was recorded for each solution, and the mean value of the absorption signal was used for subsequent calculations. The absorption signals were evaluated by subtracting the value of the blank from the signal of the sample.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research has been funded by the Research Deanship of University of Ha’il, Saudi Arabia, through Project RG-20 113. A collaborative scientific support extended by The Higher Education Commission (HEC), Pakistan, to Shahnam Shahida is also appreciated.

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