تحديد كمية الرصاص في دم الانسان والمصل ودراسة العلاقة بين تأثيراته على مكونات الدم

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ملخص: *في هذه الدراسة، تم فحص ستة أشخاص بأعمار مختلفة من سكان مدينة الزاوية لم يتم تسجيل لديهم أي مستوى للرصاص في الدم من قبل. حيث تم جمع عينة واحدة من الدم الوريدي من كل منهما. يتكون إعداد وتحضير العينة بتخفيفها (عشرين ضعف) بواسطة حمض النيتريك. تم حفظ عينات المصل و الدم في مجمد بعد فصلها باستخدام جهاز طرد مركزي ، ثم تحليلها لمعرفة محتوى الرصاص في كل عينة عن طريق جهاز امتصاص الطيف الذري .*

DETERMINATION OF LEAD IN HUMAN BLOOD AND SERUM AND THE RELATIONSHIP AMONG ITS EXPOSURES, ZAWIA, LIBYA

Abstract:

In this study, six residents of Zawia City who had no history record of occupational lead exposure were investigated. One sample of venous blood was collected from each. Sample preparation consisted of a simple dilution (twenty–fold) with 0.15N HNO_3. The sample was centrifuged to separate serum, which was then isolated and analyzed for lead content by Flame Atomic Absorption Spectroscopy (FAAS). The residue of the sample was also analyzed by the same analytical technique for blood lead levels. The blood lead level in the residue was higher than that in serum lead level. Mean lead concentrations were 4.48 µg/L in serum and 60.23 µg/L in residual blood respectively. Blood lead level was quietly correlated with serum lead level and accounted for 95% of the variability of serum lead concentrations. The bivariate regression coefficient of serum lead was 0.221 (p.0.001). In a multivariate regression model of serum lead levels that included blood and serum lead levels remained an independent predictor of lead plasma level (p.0.007,

p<O.001). Our data suggest that the concentrations of lead in residual blood are high compared to that in serum.

Keywords: blood, lead exposure, residual , serum, spectroscopy

Introduction

Lead is a toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. It is a cumulative toxicant that affects multiple body systems, including the neurological, hematological, gastrointestinal, cardiovascular and renal systems [1,2]. In blood, more than 98% of the lead are found in blood cells. Plasma lead (PbP) has an important role in lead metabolism, where it is the active center of the lead body pool, although the concentration is very low levels. PbP is equilibrated with the extra-cellular pool and is directly involved in all the movements of lead among the different biological compartments [3].

Lead is stored in the teeth and bones, where it accumulates over time. Human exposure can be assessed directly through its measurement in blood, teeth or bones (bone and tooth lead reflect cumulative exposure) [4]. The most critical effect of lead in young children is that on the developing nervous system. Subtle effects on intelligence quotient (IQ) are expected from blood lead levels at least as low as five µg/dL (50 µg/L), and the effects gradually increase with increasing levels of lead in blood [5]. At levels above ten µg/dL, it is well-established that exposure to lead can have harmful effects on some body functions and organs in both adults and children. This can include effects on blood pressure, kidneys, blood cells and the brain and nervous system. At levels above 100 µg/dL, the effects can become life threatening. The blood lead level at which people have clinical symptoms varies among individuals, with more severe effects seen at higher blood lead levels [6]. The present study aims to determine the concentration of blood Pb, and Pb serum levels of selected residents live in the different location of Zawia City, Libya. We have also investigated the relationships among its exposure, using FAAS.

Materials and methods

1. The Sampling Collection and Storage

Collection and storage of blood and serum samples for Pb analysis require special handling to avoid contamination, especially if the variables to be measured in very low concentrations. All glassware and plastic containers used for the collection and storage of samples were cleaned in a detergent solution to avoid contamination. They were also rinsed with ultrapure water Milli-Q water and soaked in a nitric acid bath (20%, v/v) overnight. This was followed by thoroughly rinsing with ultra-pure water and dried.

2. Apparatus

Atomic Absorption Spectroscopy (Varian 730 model, Germany) was used for measurement of lead concentrations in the whole blood and serum samples. A centrifuge (model 800) was used to separate the serum of blood sample. All plastic containers were made of polypropylene (PP), to minimize contamination.

3. Chemicals

All chemicals were pure analytical grade reagents, and solutions were prepared in deionized water. Concentrated nitric acid (Kimix) was used as the solvent.

4. Samples preparation and methodology

All laboratory glassware and plastic-ware for samples preparation were first cleaned with deionized water to avoid contamination, then with nitric acid 2% and rinsed again with deionized water. Some laboratory methods are available to determine blood lead concentrations [7]. The accurate and precise determination of plasma and serum lead levels depends on meticulous processing techniques, including control of lead contamination, hemolysis, and redistribution of lead due to anticoagulants, and, in the case of serum sample collection, the time interval for whole blood dotting before the separation of serum [8]. The best method of measuring lead in serum is to use ICP-MS.

Unfortunately, this was not available, and so, in this study, the samples were analyzed by FAAS (Varian 730 model, Germany).

The subjects were four men and two women (25-85 years old, mean 50 years). They live near to Zawia refinery and electricity power station, and some of them live in other places. 12 blood samples (n=12) were collected during the present study. The samples were collected in acid- washed polypropylene tubes. Venous blood was drawn from the cubital vein into an evacuated and heparinized 5 ml tube Serum was separated by centrifugation (10 min, 1,200 g) within 10 min after the sampling and transferred into acid-washed polypropylene tubes. The blood samples and serum were stored at -20°C. The analysis was carried out within three weeks after sampling. Distilled pure water (DW) was prepared by filtration of distilled water through a Millipore-Q system. For the preparation of the sample of solutions for FAAS determination, whole blood (300µl) was hemolyzed with 2.70mL of DW, then well mixed with 11.2mL of nitric acid (0.15 N). Serum (300µl) was well mixed with 11.2mL of nitric acid (0.15N).

The average of three replications was used for calculations. The instrument was calibrated against spiked serum (+10 and +20 µg/l) and whole blood (+500 and +1,000 µg/l) samples.

Results and Discussion

The results that were obtained from the experiments are presented in the form of tables and figures with some explanations. The study of Pb in blood and its serum was undertaken to understand the relationship between the exposure of metal in the human blood.

Table 1: Lead concentration in blood and its serum

Sample	Conc (µg/l)	SD (±)	Age	Location
B1	30.50	2.65	29	Zawia coastal road
B2	50.20	5.45	85	Zawia Refinery
B3	95.20	7.50	25	Alharsha Electricity Power Station

B4	43.50	7.50	35	Asabria coastal road
B5	41.50	2.50	20	Asabria farm
B6	100.50	4.50	20	Asabria pertrol station
S1	2.30	0.50	29	Zawia coastal road
S2	4.50	1.25	85	Zawia Refinery
S3	6.50	2.25	20	Alharsha Electricity Power Station
S4	6.50	1.50	35	Asabria coastal road
S5	5.50	1.20	20	Asabria farm
S6	1.60	0.50	25	Asabria petrol station

B1, Blood – S2, Serum – SD, standard deviation

In general, Pb concentrations in residual blood sample were higher than that in serum during the study period (Table 1, Figure 1). Lead blood concentration varied between 30.50 and 100.50µg/L with a mean value of 119 µg/L. Lead serum concentration also varied between 1.60 and 6.50 µg/L with a mean value of 4.48 µg/L. The highest Pb blood and serum concentration (100.50 and 95.20 µg/L may be due to emissions from Alharsha Electricity Power and Asabria petrol station in recent years. In comparison with blood guidelines, the highest Pb level seem to be close to normal blood levels of 100 µg/L [6].

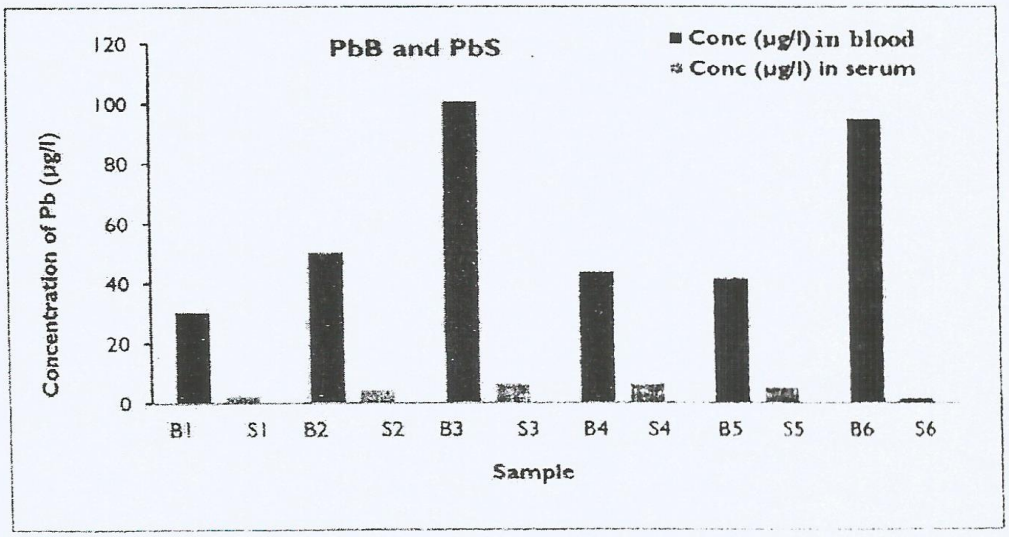

Figure 1: Concentrations of lead in blood and serum samples

Descriptive statistics for biomarkers of lead exposure are presented in Table 2. The positively correlation coefficient (1.48) between blood and serum lead levels was observed. Blood lead levels accounted for 95% of the variability observed in serum lead concentrations.

Table 2: Descriptive summary statistics for blood and serum lead levels

Count	6 blood samples	6 Serum samples
Mean	60.23	4.48
Median	46.85	5.00
SD (±)	29.87	2.11
Minimum	30.50	1.60
Maximum	101.0	7.00
Sum	361.4	26.9

When we simultaneously included blood lead and serum lead variables, blood lead was a highly significant predictor of serum lead in the model ($p<0.005$) (Table 3). The estimated regression coefficient between the log blood lead concentration and the serum lead level was 0.221.

Table 3: Bivariate regression model correlating serum lead levels with blood lead levels

Biomarker	Blood
Constant	-0.14
Coefficient	0.221
Standard Error	0.261
P-value	0.002
R^2	0.669

Conclusion

Based on the results and discussion of this study, the findings of this study may have great importance regarding public health. The applications of AAS methods to biological samples also provide relevant information about clinical chemistry, clinical toxicology, forensic toxicology as well as workplace testing or environmental exposure. One of the main reasons is the very low concentration

of Pb serum, which is difficult to determine by atomic absorption spectrometry (AAS).

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