



THE ROLE OF THE ATOMIC ABSORPTION SPECTROMETRY IN THE STUDY OF BLOOD LEAD LEVELS AND OUTLET DIALYSATE LEAD LEVELS FOR CHRONIC KIDNEY DISEASE PATIENTS

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Abstract. Some of the naturally occurring chemical elements (like Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Se, Sr and V), are essential to life but they also can cause various human problems. Along with these, Lead – as a toxic substance - can affect almost every organ and system in the body. The authors have proposed to study how the levels of this chemical element are modified for the patients with normal renal activity in comparison with the patients with chronic renal failure. In order to determine the chemical elements included in the study, the researchers used an Atomic Absorption Spectrometry in Graphite Furnace (GF-AAS). The levels of Lead have proved to be increased for the patients with chronic renal failure who underwent blood dialysis

Keywords: Atomic Absorption Spectrometry, Lead, Dialysis, chronic renal failure

Introduction

Many of the naturally occurring chemical elements are present in the body on their own or linked together to form organic and inorganic substances, and that facilitates or creates various human problems. Some of the elements (Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Se, Sr and V), are essential to life. They must be available at various levels, considered normal. These standards can be evaluated for several biological products: blood, urine, cerebrospinal fluid, nails, hair, tears or sweat, in organic products. The value levels of these organic products must be considered normal around the average for that item. In this case, both deficiency and excess of these elements generate certain problems.

Usually the deviation is due to normal metabolic diseases, but they depend also on the ratio of daily intake and quantity of removed items.

Other elements (As, Ba, Be, Cd, Li, Hg, Pb, Sb,

Sn) are considered toxic and it should be desirable that the value of their concentration in any biological product to be zero. When this concentration is different from zero, values are considered normal if lower than the limit values of the local toxicology standards. Contamination or poisoning due to these factors is due to the environment or food. Some of these rules are described in laws concerning exposed working people.

Elimination from the body of the essential elements as well as the toxic substances is performed mainly by urine flow [1-3]. For chronic renal failure patients, this removal is performed only during dialysis sessions. The authors have proposed to study how the levels of lead are modified for the patients with normal renal activity in comparison with the patients with chronic renal failure. In order to determine the chemical elements included in the study, the researchers used an Atomic Absorption Spectrometry in Graphite Furnace (GF-AAS).

Atomic Absorption Spectrometry measures the amount of light absorbed by a sample that contains an item brought in a state of atomization. The amount of absorbed light is directly proportional to the concentration of that element for the analyzed sample [4].

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Material and methods

This study aims to prospectively analyze two groups:

- A group of 36 patients (22 men, 14 women) with chronic kidney disease - blood samples and outlet dialysis samples have been analyzed;
- A group of 50 persons (37 men, 13 women) with no disturbances of the renal function - control group – blood samples have been tested.

For this study, the including criteria of the patients were related to age, sex and renal function.

Including criteria for the control group are:

- Normal renal function
- Age between 3 - 60 years
- Subjects must belong to the local habitat

Including criteria for the examined group of patients are:

- Patients with chronic dialysis in a health facility
- Age between 18 - 65 years
- Dialysis efficiency evaluated by $kt/V > 1.2$ formula (k – dializer clearance of urea ml/min; t – dialysis time min; V – volume of distribution of urea ml).

Control group persons and patients in the study group will be divided according to sex type. For the purpose of the study, the following data will be recorded:

- from the control group: a sample of 1 ml blood, taken on an heparinized test tube and blood biochemistry vacutainers for serum creatinine evaluation;
- from patients: a sample of 1 ml blood, taken on an heparinized test tube before dialysis, and a sample of 1 ml blood at the end of it. Also a sample of 10 ml of outlet dialysate is taken.

To determine the elements for this study, a device of Atomic Absorption Spectrometry with Atomization in Graphite Furnace has been used - GF-AAS [5].

Equipment

AAS device – Varian – consists of:

- Atomic Absorption Spectrometer SpectrAA 880;
- Orchard GTA 100 graphite furnace
- 25 PSD autosampler
- CFT watercooler 33 – Neslab
- nitrogen generator - dominikhunter (purity 99.999%)
- Analytical Nitrogen - 99.999% purity
- Argon analytical - 99.999% purity
- equipment and fixed assets in a common laboratory of toxicology
- AAS-specific reagents and consumables.

Samples preparation

The outlet dialysate processing method consists in adding of 1000 μ l 65% nitric acid. After 20 minutes the liquid is spun for 10 minutes at 2000 rpm. The supernatant is the matrix of the injection plant. Water used for dialysis was prepared after the same procedure.

The blood processing method consists of mixing 200 μ l blood with 800 μ l antifoam B 5% and 1000 μ l water 1.6N HNO₃. After 20 minutes the liquid is spun for 10 minutes at 5000 rpm. The supernatant is the matrix of the injection system [6].

Work parameters

To determine the lead, the system operates with the following program:

- pipetting mode - automix
- measuring mode - peak level
- calibration - in concentration
- smoothing - 9 points
- number of replicate - 2 standard and 2 samples
- lamp current – 10 mA
- wavelength – 283.3 nm
- slit width – 0.5 nm
- background correction - deuterium lamp
- drying – 95 – 120°C
- calcination – 400°C
- atomization – 2100°C
- 4-point calibration curve – 0,1, 3 and 5 μ g/l
- recalibration rate –16

Automatic quality control - dispersion < 10%, correlation coefficient > 0,998, RDL 0,08; IDL 0,04 [6].

For a group of 10 patients, processing (training) samples takes about three hours. Matrices for injection are placed in the carousel with samples. GF-AAS system can measure the lead concentration from 30 samples in about four hours.

Quality control of results is automatically imposed as a reproducibility better than 10%. New Rational calibration curve is obtained after the relationship (1):

where:

$$A/C = a + b * A + cA^2 \quad (1)$$

- A - sample absorbance
- C - concentration of sample
- a, b, c, d - coefficients of calibration curve

Correlation coefficient as required for automatic control is better than 0.998. Four-point calibration curve is shown in figure 1.

It worked with the rearrangement of one of the calibration curve standards. Recalibration of the system after 12 determinations was the chosen option.

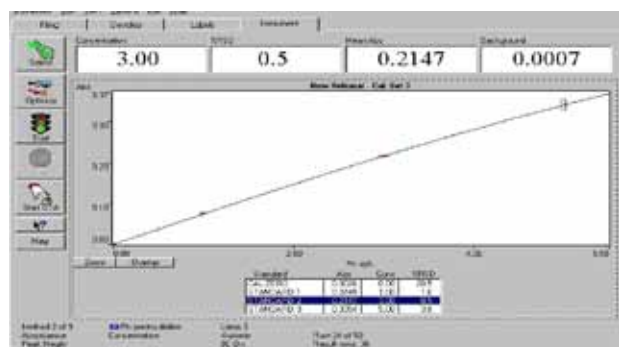


Figure 1. Calibration curve of lead

Results and discussion

After analyzing blood samples the following values were obtained for the control group - table 1.

Average lead concentration for the control group's blood sample is 3.54 µg/dl, with a standard deviation of 0.932, which shows many homogenous values. Lead concentration is well below the maximum allowed of 40 µg/ l [5].

The average age of the control group is 39.82 years with a standard deviation of 13.06 and a standard median deviation of 1.85 which shows a large distribution of values which are in the range 3-60 years.

For the control group, the average creatinine was 0.931 mg/dl, with a standard deviation of 0.221 and a standard median deviation of 0.031 which shows a normal renal function for control group subjects. Limit values of creatinine were 0.56 - 1.5 mg/dl.

For the control group, the mean lead concentra-

Number	Name	Age	Gender	Creatinine (mg/dl)	Lead (µg/dl)
1	PP	29	B	0.8	3.13
2	AA	44	B	0.9	3.66
3	CV	23	B	0.7	1.86
4	GL	41	F	0.6	2.01
5	MJ	42	F	1.2	2.04
6	ND	57	B	1	5.13
7	CG	60	B	0.9	2.09
8	MT	57	B	0.85	2.59
9	ZC	23	B	0.95	2.34
10	Unknown	60	B	0.8	4.10
11	RM	57	B	1.4	1.73
12	DC	42	F	1.18	1.85
13	BM	45	F	1.3	2.11
14	Unknown	47	F	0.95	3.80
15	IC	60	B	0.8	1.35
16	FC	27	F	0.7	1.57
17	PM	56	B	0.6	2.13
18	VA	51	F	1.2	1.49
19	TA	23	B	0.9	2.48
20	Unknown	30	B	0.9	3.21
21	BV	32	B	0.7	6.13
22	MG	43	B	0.7	6.65
23	MF	45	B	0.56	1.92
24	ME	33	F	1	1.37
25	MS	41	B	1.12	3.53
26	PV	53	B	1.1	4.25
27	CE	51	F	0.88	2.19
28	DS	44	B	0.9	2.02
29	DA	46	F	0.9	2.00
30	CM	32	F	1	0.39
31	LA	27	B	1.2	3.09
32	EMD	34	B	1.5	1.98
33	SM	22	F	1.1	0
34	CC	42	B	1.4	0.37
35	DO	40	B	0.8	2.18
36	SM	38	B	0.9	1.18
37	IA	22	B	0.7	0
38	SA	59	B	0.66	6.56
39	AM	25	F	0.9	5.34
40	SRC	25	B	1.2	0
41	BI	54	B	0.6	16.2
42	VA	56	B	0.8	29.05
43	MG	28	B	0.9	0.37
44	MS	41	B	1	8.87
45	PM	23	B	1.1	3.48
46	Unknown	33	B	1.1	8.39
47	NA	3	B	0.8	0.26
48	MN	42	B	0.8	0
49	AG	45	B	0.8	4.8
50	IM	38	B	0.8	4.0
Average		39.82		0.931	3.54

Table I. Values of creatinine and blood lead concentrations in the control group

tion was 3.54 µg/l. Values distribution is very high - between 0 and 29.05 µg/l.

Lead concentration for the outlet dialysate fluid was 0.05 µg/l.

After blood sample analysis before (A) dialysis, after (B) dialysis and from dialysate (LD) for patients group, the following values were obtained – table 2

cases the Cochrane threshold had a value of 1.96.

Outlet dialysate mean concentration was 15.38 µg/l with values between 2.35 and 69.0 µg/l. Because of the large range of values, the standard deviation was 17.14 and the standard deviation of median was 2.86 [7,8].

Corrected value of median lead concentration

Sample type	Patient								
	1	2	3	4	5	6	7	8	9
A	6.21	7.51		9.11	6.78	9.2	4.27	8.12	6.69
B	7.65	8.26	7.26	9.22	5.86	7.76	5.41	6.99	7.37
Dialysate	10.1	9.07	2.65	9.22	2.35	2.75	3.03	4.88	3.64

Sample type	Patient								
	10	11	12	13	14	15	16	17	18
A	6.04	6.02	25.2	17.6	16.5	13.1	17.8	15.6	15.0
B	5.88		7.64	7.43	7.7	4.92	11.0	5.96	6.15
Dialysate	22.8	18.1	27.3	39.2	26.9	28.2	25.4	66.5	69.0

Sample type	Patient								
	19	20	21	22	23	24	25	26	27
A	4.96	4.67	14.0	4.4	3.85	4.08	5.45	5.75	12.2
B	4.99	6.05	4.24	3.99	6.49	4.22	7.76	8.37	5.86
Dialysate	33.1	43.1	23.7	4.20	6.05	8.93	4.58	4.87	5.37

Sample type	Patient								
	28	29	30	31	32	33	34	35	36
A	4.680	3.85	2.60	3.19	4.01	3.76		3.62	2.87
B	8.49	3.67	2.9	7.68	4.57	3.75	2.57	3.4	3.24
Dialysate	3.35	4.33	6.07	6.12	6.59	5.85	5.75	5.28	5.46

Table II. Blood lead concentration values before the dialysis procedure (A) at the end (B) and outlet dialysate (LD) in the group of patients (blood - µg/dl; dialysate - µg/l)

Student test applied to the hair and blood concentration of lead in the samples of patients before dialysis (A) and restitution (B) showed a mean difference of 1.95 lots µg/dl:

Student test applied for lead blood concentration for the patients group before dialysis (A), after dialysis (B):

- average blood levels before dialysis was 8.04 µg/dl
- average blood levels after dialysis was 6.08 µg/dl
- average concentration in the dialysate was 15.12 µg/l

The result of statistical processing of lead blood level in patients group with chronic kidney disease shows that the average amount removed by dialysis was 1.95 µg/dl. The difference between the average blood levels before and after dialysis is statistically considered as significantly different from zero, for a probability below 0.05 with a Student threshold of 1.95 for a T = 2.25.

Compared to the control group, the mean lead blood levels for dialysis patients were significantly higher with a probability p < 0.01, both at the initiation - 4.5 µg/dl (for T = 3.94) and also at the end of the procedure - 2.53 µg/dl (for T = 3.45). In both

in the outlet dialysate is 15.33 µg/l.

For all dialysis procedures, the dialysate flow was 500 ml/min.

Determining the lead concentration dialysate allows counting the total amount of the removed Lead through dialysis procedure. The total amount of removed lead was 2.07 mg Pb per session. Lead clearance by the dialysis procedure is 7.665 µg/min.

Lead clearance in the peritoneal dialysate fluid related to the average of blood concentration at the end of dialysis procedure is (2):

when

$$Cl_{Pb} [l/min] = \frac{C[\mu g/l] * D[l/min]}{P[\mu g/l]} \quad (2)$$

- C - Lead concentration in outlet dialysate
- D - dialysate flow
- P - blood lead concentration at the end of dialysis procedure

Calculating, the clearance value can be obtained related to 124 ml/min blood concentration.

Dialysis procedure time was 4.5 hours.

In this case, the average amount of removed lead

by the dialysis procedure is 2.07 mg Pb per session.

Absorbance signals of dialysate, and two blood samples taken from dialysed patients are shown in Figures 2-4.

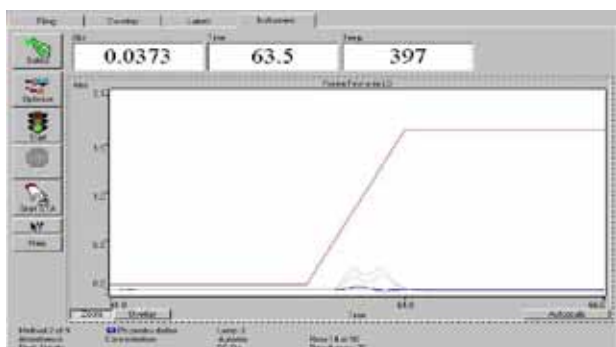


Figure 2. Absorbance outlet dialysate signal of patient no.1

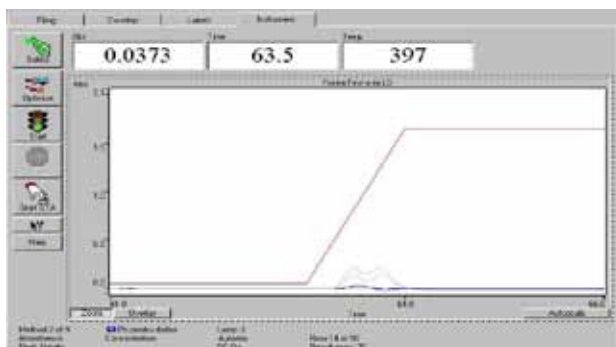


Figure 3. Absorbance blood signal before the dialysis of patient no.1

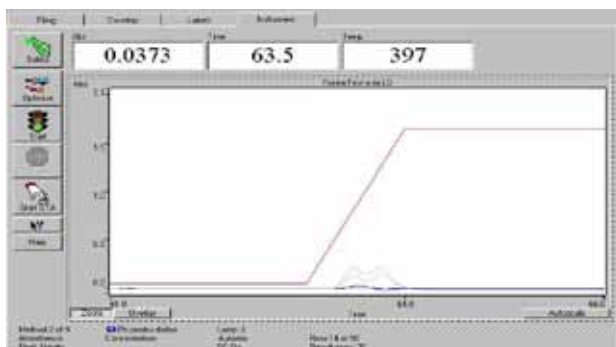


Figure 4. Absorbance blood signal after the dialysis of patient no.1

Conclusions

The study provides very useful information over these essential and toxic elements in the blood of patients with chronic renal failure in comparison with persons with normal renal function. Analytical diagnosis of certainty is established by Atomic Absorption Spectrometry and Graphite Furnace Atomization. The method is highly sensitive and reproducible but the whole procedure requires highly skilled personnel.

Methods of preparing arrays of injection are simple and offer a very good digest of their non-anatomic removing pads that may occur during the test.

The graphite furnace temperature ensures a very good drying and calcinating of the samples, without loss of item, although lead is part of the volatile elements.

The sensitivity and detection limit of the method are very good and very precise information is given by the results. Reproducibility was, on average, better than 5%.

As a result of dialysis, the average lead concentration decreased by 1.95 $\mu\text{g}/\text{dl}$ significantly statistics by Student Test.

Comparing with the control group (average of 3.54 $\mu\text{g}/\text{dl}$), the mean concentration of lead, both at the beginning of the procedure (average 8.1 $\mu\text{g}/\text{dl}$) and at the end of the procedure (average 6.14 $\mu\text{g}/\text{dl}$), was decreased – statistically significant – more for patients with chronic renal failure undergoing dialysis than for persons with normal renal function.

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