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DEVELOPMENT OF A METHOD FOR ALUMINIUM DETERMINATION IN SERUM AND DIALYSIS FLUID BY FLAMELESS ATOMIC ABSORPTION WITH GRAPHITE FURNACE

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ABSTRACT

Aluminium determination was carried out in serum and dialysis fluid by a simple and reliable method of flameless atomic absorption with graphite furnace., No preparatory procedures are required for water and dialysis fluid. In this method serum was mixed with 0.2% HNO_3 to allow complete combustion of the samples in order to improve analytical precision. The method has a sensitivity of 15 pg and detection limit of 2.1 µg Al/L.

The aluminium contents of the main water supply, be-distilled water and dialysis fluid were 52, 1, and 44 µg/L respectively. The mean value for normal serum aluminium in Isfahan was 3.6 µg/L. Serum aluminium concentration in chronic renal failure was measured in pre- and post-dialysis samples. The mean values for the serum aluminium levels pre- and post-dialysis were 30.5 and 71.08 µg/L with a range of 9-60 and 21-123 µg/L, respectively. Pre- and post-dialysis serum aluminium values in female patients were within the range of 1-42 and 12-66 µg/L with mean values of 18.25 and 40.5 µg/L, respectively. Instrumental settings and sample handling are discussed.

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INTRODUCTION

Patients with chronic renal failure on regular hemodialysis were reported to suffer from a special intoxication due to high levels of aluminium in blood and tissue.[1] Dialysis dementia, hypochromic microcytic anemia, dialysis osteomalacia and Alzheimer's disease are the effects of this intoxication.[2,3,4] Contamination of dialysis fluid with aluminium and/or intestinal absorption of aluminium from aluminium phosphate binders in those who use these agents to prevent hyperphosphatemia are both sources of aluminium accumulation.[5,6] Aluminium from either source enters the blood circulation and binds to transferrin,[7] a β-glycoprotein responsible for iron transport.[8,9] The binding of aluminium to serum transferrin and its interaction with iron metabolism might be the cause of anemia in these patients,[10-12] but the exact mechanisms by which other disturbances occur in these patients are still unknown and no evidence is available

233

in the literature. However, in order to protect these patients from developing aluminium toxicity, it is necessary to monitor the aluminium content of the water used for the preparation of dialysis fluid and also to monitor the aluminium content of the dialysis fluid. The method used should also be able to measure aluminium in serum and other biological fluids and tissues. A number of techniques including neutron activation analysis (NAA), inductivity coupled plasma (ICP), etc. have been reported for the measurement of aluminium in serum.[13-15] These methods are neither accurate nor available in all laboratories for routine aluminium determination due to the cost of the necessary equipment and instruments.

In this paper, we describe a method using flameless atomic absorption with graphite furnace which is suitable for routine aluminium determination in serum and other biological fluids. The technique requires minimal sample preparation and provides adequate precision and accuracy.

MATERIAL AND METHODS

All chemicals were reagent grade and obtained from Sigma Chemical Company. Throughout this study be-distilled water with an aluminium content of less than 1 μg/ L was used. To minimize metal contamination, all glassware was soaked overnight in 20% nitric acid and washed three times with be-distilled water. Plastic containers were washed with 1 mM EDTA to remove metal contamination and washed three times with be-distilled water. All pipette tips were cleansed by dispensing two volumes of 10% HNO_3 and three volumes of be-distilled water.

Sample collection
Blood samples were collected from hemodialyzed patients in Shariati Hospital Dialysis Center, and also from normal controls by standard venipuncture. The blood samples were transferred into 10 mL pre-cleaned plastic centrifuge tubes. After centrifugation the sera were transferred into 5 mL plastic sample tubes and either used immediately for aluminium determination or stored in a refrigerator at -10°C.

Instrument conditions for serum Al determination
Aluminium determinations were carried out using a Perkin-Elmer atomic absorption spectrophotometer (Zeeman-3030) equipped with an HGA-600 graphite furnace, an automatic sampler and a Perkin-Elmer recorder. The autosampler was able to deliver 20 μL aliquots of sample and standard solutions.

Temperature program selection
The temperature program for aluminium determination was set as follows: three stages were selected in order to measure aluminium in serum and aqueous solutions. These stages are named as drying, ashing and atomizing. The instrument was set as follows:

Step	Stage	Temperature (C°)	Hold (S)	Ramp
1	Dry	90	10	4
2	Dry	300	10	8
3	Ash	1500	7	10
4	Atomize	2500	2	0

Wavelength was 309.3 nm, aluminium hollow cathode lamp current was 25 mA, and the spectral band width was 0.7 nm. Argon gas was used as inert gas.

Preparation of calibration curve
Aluminium working standards containing 0-60 μg/L were prepared from a commercial 1 mg Al/mL stock solution (specially made for use in atomic absorption) by dilution with be-distilled water containing 0.2% HNO_3. The stock solution was shown to be stable for a minimum of one year and working standards were stable for at least two weeks.

A typical standard curve is shown in Fig. 1 in which each point is the mean of ten separate measurements.

Serum pre-treatment
Serum samples from either controls or hemodialyzed patients were mixed with an equal volume of 0.2% HNO_3 to eliminate the problems of organic residue accumulation in the furnace. The mixture was vortexed well and placed in the instrument for aluminium analysing.

RESULTS

Establishment of the method
Preliminary experiments were carried out to calculate the detection limit and the sensitivity of this method.

The detection limit, defined as that concentration of aluminium which gives an absorbance signal equal to twice the standard deviation of the background signal from a reagent blank was found to be 2.1 μg for serum. The characteristic concentration (that amount of element which gives an absorbance of 0.0044) was 15 pg.

The next experiment carried out was to calculate the percentage of recovery of the method. To approach this, 100μL of 20 μg Al/L standard solution were added to a series of serum samples with known aluminium concentration.

The aluminium concentrations of sera were then determined and the percentage of recovery calculated. The results obtained are presented in Table I. This illustrates the results of a standard addition to 6 serum samples.

Recovery of 86-108% of added aluminium indicated that sera diluted 1:1 with 0.2% HNO_3 can be used for aluminium determination with such instrumental conditions.

234

Table I. Results of recovery of aluminium added to serum samples

Sample No.	Aluminium before addition ng/mL	Amount added ng/mL	Amount found ng/mL	% Recovery
1	1	20	10	90
2	2	20	11	100
3	6	20	14	108
4	6	20	14	108
5	2	20	11	100
6	1	20	9	86

1 mL of Al solution containing 20 ng/mL was added to the serum sample. Total volume was 2 mL. Aluminium determination was then carried out in the 2 mL solution.

Aluminium determination in serum and dialysis fluid

Following establishment of the method, the aluminium content of water supply, be-distilled water and dialysis fluid were determined. The aluminium content of each was 52, 1 and 44 µg/L respectively. The high concentration of aluminium in dialysis fluid might be due to the contamination of the water used for the preparation of this fluid. The aluminium content of the concentrated dialysis fluid was less than 1 µg/L.

Serum aluminium concentrations of 16 chronic renal failure patients were determined. The results are presented in Table II.

As can be seen, in controls serum aluminium concentrations were within the range of 1-9 µg Al/L with a mean value of 3.62 µg Al/L, whereas the serum aluminium concentration of renal failure patients was within the range of 11-132 µg Al/L, with a mean value of 46.75 µg Al/L, revealing a high concentration of aluminium in the sera of these patients.

Pre- and post-dialysis serum aluminium determination in hemodialyzed patients

Serum aluminium concentrations were determined prior to and after dialysis in 16 male and 6 female patients on regular hemodialysis. The pre-dialysis serum aluminium concentrations in male patients were within the range of 9-60 µg Al/L with a mean value of 30.5 µg Al/L, whereas post-dialysis serum aluminium concentrations in the same group were within the range of 21-123 µg Al/L with a mean value of 71.8 µg Al/L. It is obvious that a significant elevation of aluminium occurs in the serum of patients following hemodialysis (Table III).

The aluminium concentrations of the sera of 4 female patients were also determined. The pre-dialysis serum aluminium concentration in these patients was within the range of 1-42 µg Al/L with a mean value of 18.27 µg/L and the post-dialysis serum aluminium concentration was within the range of 12-66 µg /L with a mean value of 40.5 µg Al/

Table II. Aluminium concentration in 16 normal and 16 chronic renal failure (CRF) patients. Each value is the mean of three measurements.

No.	µg Aluminium per liter	
	Normal	Renal failure
1	1	21
2	3	30
3	1	30
4	1	114
5	9	69
6	3	20
7	9	21
8	4	75
9	2	33
10	7	60
11	1	81
12	2	12
13	6	18
14	6	11
15	2	132
16	1	21
Mean	3.62	46.75
Range	1-9	11-132

Table III. Pre- and post-dialysis measurement of serum aluminium in 16 male patients with chronic renal failure.

No.	µg Al/L	
	Pre-dialysis	Post-dialysis
1	42	57
2	15	45
3	60	75
4	54	96
5	30	114
6	30	66
7	24	69
8'	9	51
9	45	123
10	18	36
11	21	78
12	10	21
13	10	60
14	39	93
15	30	81
16	51	84
Mean	30.5	71.81
Range	9-60	21-123

L. The data are presented in Table IV. It appears that serum aluminium concentrations were higher in men than in women with renal dysfunction.

235

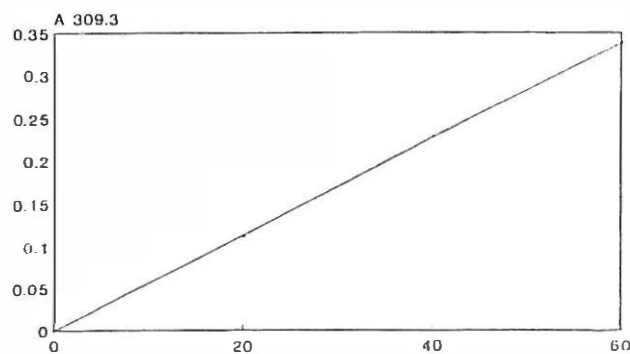Fig 1. Aluminium (mcg/L)

Table IV. Pre- and post-dialysis measurement of serum aluminium in 4 female patients with chronic renal failure.

No.	μg Al/L	
	Pre-dialysis	Post-dialysis
1	42	51
2	12	33
3	18	66
4	1	12

Mean   18.25   40.5
Range 1-42   12-66

DISCUSSION

The association between aluminium accumulation in uremic tissues and the development of dialysis encephalopathy, osteomalacia, and hypochromic microcytic anemia require patients on regular hemodialysis to he monitored frequently. The most important source of aluminium toxicity is the water supply which is used for the preparation of dialysis fluid.[6] The aluminium content of this water should be checked since the efficiency of the purification technique may vary from day to day.

The technique described here allows reliable determination of aluminium in serum and aqueous solutions. In this method, using 10% HNO_3 for glassware and 1 mM EDTA for plastic containers can prevent the problem of contamination since no detectable aluminium was found by comparing the absorption signals obtained from fresh sera and water samples with those obtained from samples held in the containers (data not shown). The temperature stages used led to the complete atomization of aluminium and produced a sensitivity and detection limit of 15 pg and 2.1 μg/L, respectively. Parkinson et al.[16] used flameless atomic absorption (Perkin-Elmer 603 spectrophotometer) and found a sensitivity and detection limit of 35.5 pg and 2.3 μg/L, respectively. Our findings are in good agreement with their

observations. Obviously, the sensitivity produced by our instrument was much better, due to the atomic absorption model which was much more modernized. In the present method the linearity of our calibration curve was up to 60 ng/mL of aluminium. With such a calibration curve we were able to measure aluminium concentrations in serum, although the serum should be diluted in higher levels of aluminium. Mazzeo-Farina and Cerulli have reported a linearity of up to 50 ng/mL which was in agreement with our findings.[17]

The results obtained from serum aluminium concentrations in normal sera were within the range of 1-9 μg Al/L with a mean value of 3.62 μg Al/L in Isfahan. The normal value was found to be much lower than reported by Parkinson et al.[16] (7.3 μg Al/L) or Day which was less than 5 μg/L.[18] These variations could be either due to the method of serum sample collection or the geological condition of the living area. The high concentration of aluminium in hemodialyzed patients is due to the transportation of aluminium from the dialysis fluid. We found that the aluminium concentration in the water supply used for the preparation of dialysis fluid was very high and it could be replaced with be-distilled or deionized water. We recommend that the amount of aluminium in dialysis fluid should be less than 10 μg/L for all dialysis centers in Iran.

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236

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237