ORIGINAL SCIENTIFIC PAPER

ALUMINIUM IN WATER FOR PREPARATION OF DIALYSATE AND IN SERUM OF DIALYSED PATIENTS*

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Received April 23, 1997

The aim of this study was to develop and verify the electrothermal atomic absorption method for aluminium determination in human serum and correlate the concentration of aluminium in serum of patients on dialysis and in water for preparation of dialysate. Two centres were included: centre A with accidentally enhanced concentration of aluminium in water for preparation of dialysate and centre B with very low water aluminium. Aluminium level in serum of healthy people was also analysed. The results showed that the analytical method was reproducible and sufficiently accurate in determining serum and water aluminium. Normal values obtained for aluminium in the sera of healthy people ranged from 0.9 to 12 μg/L and were significantly lower than all values obtained from the dialysis centre A. The aluminium concentration in serum of dialysed patients displayed linear correlation to aluminium concentration in water used for preparation of dialysate.

Key terms:
aluminium determination, dialysis, electrothermal atomic absorption spectrometry, serum aluminium

Although the third most prevalent element in the world, aluminium in serum of normal human population is extremely low. Due to efficient skin, lung and gastrointestinal tract barrier, serum aluminium is about 1–10 μg/L. Aluminium is allowed to be added to municipal water supplies, to processed food and medications. Unusual circumstances, however, may lead to an increase in tissue aluminium levels producing several toxic effects such as encephalopathy, osteodystrophy and anaemia. If parenterally administered, for example, aluminium may exert serious systemic toxicity for humans. Dialysis dementia or dialysis encephalopathy was seen in a large number of patients with renal failure undergoing chronic dialysis. Such toxic effects have been noticed and ascribed to aluminium ever since the mid 1970-ies. The disease was found in patients treated with dialysate that contained high aluminium levels (1, 2).

*The results of this study were partly presented at the "Symposium on Significance of Water Quality in Hemodialysis", held in May 8–10, 1997, Makarska, Croatia.

The purpose of this study was to: a) develop and validate the method of aluminium determination by electrothermal atomic absorption spectrometry; b) estimate the concentration of aluminium in serum of normal population and c) correlate aluminium concentration in treated water used for dialysate preparation and in serum of patients dialysed in two dialysis centres in Croatia.

SUBJECTS AND METHODS

Aluminium determination

Electrothermal atomic absorption spectrometry (ET AAS) procedure with magnesium nitrate as matrix modifier was applied to quantify aluminium in water for preparation of dialysis solution and in the serum of normal people and patients on dialysis (3–6). Varian Spectraa 300 with Graphite Tube Atomizer GTA 96 (pyrolytically coated graphite tubes) and Epson™ FX-850 printer was used. The new polyethylene ware, used for sampling and sample preparation for ET AAS measurement, was tested for aluminium contamination by adding 3 ml of 0.6% HNO_3, that were subsequently mixed and analysed. Since aluminium contamination was found even in the new tubes, we applied the following washing procedure: soaking in 16% HNO_3, rinsing in deionised water, repeated soaking in 3% Na_2EDTA and final rinsing with deionised water of high purity (electroconductivity – 0.06 μS/cm). Aluminium Spectrosol standards of 1,000 ppm Al (BDH Chemicals Ltd. Pool, England) were diluted with 0.6% HNO_3 (Suprapur, Merck, Germany) for measurements. The modifier used for ET AAS measurements consisted of a 0.17% solution of $Mg(NO_3)_2$ x $6H_2O$ (pa, Kemika, Croatia) prepared with an addition of 0.5% of Triton-X100 (purified for LSC, Packard Instrument Co., Ltd., USA). The reference standards for the method validation were the human albumin BRP (European Pharmacopoeia, Council de l'Europe, France) and Seronorm™ Trace Elements Serum (Nycomed Pharma AS, Norway). Deuterium correction and the standard addition method during ET AAS measurements were found unnecessary, except when normal values of aluminium in serum were measured. The temper-

Table 1 *Furnace temperature programme for electrothermal atomic absorption spectrometry of aluminium in serum or water*

Temperature (°C)	Time (sec)	Gas flow (L/min)	Gas type	Read command
85	5.0	3.0	Nitrogen	no
100	15.0	3.0	Nitrogen	no
125	15.0	3.0	Nitrogen	no
1200	12.0	3.0	Argon	no
2530	3.0	0.0	Argon	yes
2530	1.0	3.0	Argon	no
40	12.5	3.0	Nitrogen	no

ature programme (Table 1) was carried out with nitrogen as an inert gas at lower temperatures and with argon during ashing and atomisation stage. The sampler parameters were: 15 μL of sample and 5 μL of the modifier. Measurements were carried out at 309.3 nm wavelength, in the linear range of the calibration curve, that is, between 5 and 20 μg Al/ L, in the peak height mode. All ET AAS measurement parameters for serum were set to be equal to the water parameters. When necessary, sample dilutions were prepared with 0.6% solution of HNO_3.

Patients and sampling

In order to obtain normal values of aluminium in serum, blood samples were collected from fifty healthy subjects of both sexes, from 25 to 56 years of age, with no recorded drug intake. Serum samples were separated after coagulation and centrifugation of collected blood at 3,000^0/min and stored at -20 °C until analysis.

Purified water and serum of patients from two dialysis centres (A and B) were evaluated for aluminium on several occasions for two years, from 1995 to 1997. At the time, the war circumstances in the area producing damage to the dialysis centre, the saturated reverse osmosis membrane, and the municipal water treatment with aluminium sulphate largely affected the aluminium intoxication of the patients from centre A. Clinical symptoms indicated central neurological disorders with difficulties in speech, change in personality, seizures and dementia. Diffuse bone pain and spontaneous fractures were also noted (7). In September 1995, the first blood sampling for aluminium analysis included nine patients. After the replacement of reverse osmosis membrane, the sampling was repeated on three occasions during 1996 and 1997 with 50 to 68 patients at each sampling. Only four patients from the first sampling were followed up throughout the study. Before the first blood sampling, the patients had been on haemodialysis from two months to 16 years (3.6 years in average). In centre B, the water was correctly purified throughout the monitoring period. The blood was collected twice; the first time at the end of 1995 and the second time a year later. The first group of 21 patients had been on haemodialysis from four months to 15.5 years (4.5 years in average). The second collection included 16 patients from the same group who continued haemodialysis treatment in the same centre.

No patient in any centre had been treated with aluminium phosphate binders or with antacids for at least two years. Deionised water used for preparation of dialysate was collected for aluminium analysis at each sampling and in both dialysis centres.

Statistical procedure

The data are presented as arithmetic means and standard deviations of the mean. The within-day precision of the analytical method was calculated as coefficient of variation (expressed in percentage) after 10 repeated measurements of the same solution. The between-day precision was obtained by measuring freshly prepared solutions each day for 10 days. Linear correlation and the differences between groups of dialysed patients were evaluated by Duncan-s multiple range test (at the level of $P<0.05$) using Statistica for Windows programme (StatSoft®, release 4.0).

RESULTS

The accuracy of the method applied for aluminium determination was tested by measurements of certified BRP human albumin and Seronorm reference standards. The obtained recovery was 105% and 98% of the declared reference value, respectively (Table 2). The precision of the tested method was 3.5% between day and 5.6% within day for BRP human albumin and 8.6% and 9.0% for Seronorm, respectively. The detection limit, calculated with three standard deviations, was 0.9 μg Al/L and the characteristic mass was 3.4 pg aluminium.

Table 2 *Verification of the method (ET AAS) for aluminium determination in human serum and water by measuring certified reference materials*

	Seronorm™	Human albumin BRP
CV within day (%)*	9.0	5.6
CV between day (%)*	8.6	3.5
Al reference value (μg/L)	61	230
Al found (μg/L)**	60.2 ± 5.2	257.9 ± 13.0

* Coefficient of variation (SD as percentage of mean) of 8–10 measurements
** Arithmetic mean of 10 measurements ± standard deviation

The new plastic tubes used for collection of water and serum samples were tested for aluminium contamination. Six randomly selected tubes from the new batch displayed aluminium concentrations from 2.6 to 12.5 μg/L. As a consequence, the new plastic tubes were subjected to the washing procedure described above.

The normal aluminium values obtained for a group of 50 healthy people were 4.5 ± 2.8 (mean ± SD) ranging from <0.9–12.2 μg/L. The obtained data displayed normal distribution as presented in Figure 1.

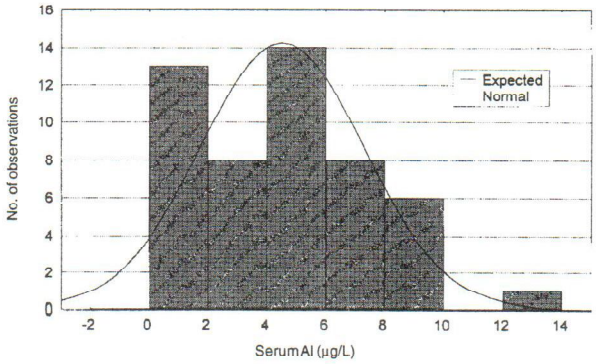

Figure 1 *Normal distribution of serum aluminium in a group of 50 healthy subjects*

Table 3 *Aluminium in water for preparation of dialysate, in serum of healthy subjects and in patients on dialysis*

		Al in water (μg/L)	Al in serum (μg/L) Mean ± SD (N)*	Range
Normal values		-	4.5 ± 2.8 (50)[a]	<0.9-12.2
Centre A	Nov. 1995.	125	140 ± 54 (9)[e]	88-236
	Apr. 1996.	8	30 ± 30 (50)[bc]	<0.9-95
	Dec. 1996.	27	75 ± 38 (50)[d]	22-174
	Mar. 1997.	<0.9	39 ± 30 (68)[c]	7-134
Centre B	Dec. 1995.	<0.9	19 ± 22 (21)[ab]	<0.9-79
	Jan. 1997.	<0.9	19 ± 11 (16)[abc]	<0.9-35

* The results are presented as arithmetic mean ± standard deviation, the number of subjects is presented in parentheses. Statistically significant differences (at P<0.05 by Duncan's multiple range test) are indicated by different characters in superscript.

At the beginning of the monitoring period in centre A when the symptoms of aluminium intoxication in patients on dialysis were noticed, the value of aluminium concentration in water used for preparation of dialysate was 125 μg/L (Table 3). Serum mean concentration of nine patients was 140 ± 54 μg/L, ranging from 88–236 μg/L. After partial servicing of the same equipment, deionised water and the serum of the patients were collected at the beginning and at the end of 1996. The obtained aluminium values respective of the sampling period were 8 and 27 μg/L for water, and 30 ± 30 (<0.9–95, N=50) and 75 ± 38 (22–174, N=50) for serum. During January 1997, a new water purification equipment was installed in the same centre after which the aluminium concentration in the water dropped to <0.9 μg/L, while the corresponding serum aluminium displayed as much as 39 ± 30 (7–134, N=68). The water for dialysate preparation in centre B was below the detection limit of the method at both samplings, while the corresponding serum values were 19 ± 22 (<0.9 –79, N=21)

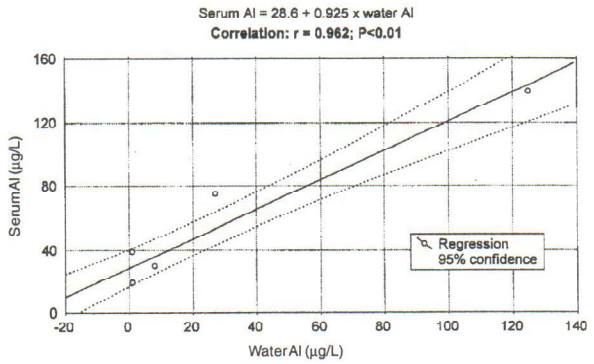

Figure 2 *Linear correlation and 95% confidence interval between aluminium concentration in water for preparation of dialysate and concentration of aluminium in serum of patients on dialysis*

and 19 ± 11 μg/L (<0.9–35, N=16), respectively. While the mean values of aluminium in serum of patients in centre B were not significantly different from the normal values, all values in centre A were significantly higher. Furthermore, the two highest mean values of serum aluminium in Centre A were significantly different from all other values (Table 3). The relation between mean aluminium values in serum and aluminium in water for dialysate preparation obtained in centres A and B are presented in Figure 2. The diagram shows a linear relationship with the starting concentration of about 30 μg/L of serum aluminium in dialysed patients when low aluminium water was used for preparation of dialysis solutions. The correlation coefficient (r) was 0.962 and it was statistically significant at the level of $P<0.01$.

DISCUSSION

By comparing different analytical methods for aluminium determination like neutron activation analysis and inductively coupled plasma with ET AAS, *Woolfson and Gracey* (8) concluded that the latter is the most suitable technique if adequate furnace programme and matrix adjusters are used. At present, ET AAS is the most widely used method for aluminium determination in human serum. The presented study achieved very good recovery and precision of ET AAS method by using magnesium nitrate as the matrix modifier, without applying deuterium background correction. While developing the method, it was noticed that particular precaution has to be taken to prevent contamination with environmental aluminium, that the glassware should be avoided and that all (even new) plasticware should be adequately washed before use. It was found that new plastic tubes contain aluminium at the level of normal human serum. Our method's detection limit of 0.9 μg/L and the sensitivity of 3.4 pg/0.0044 absorbance is comparable to similar ET AAS methods described and used elsewhere (9–11).

Normal values (baseline data) of aluminium concentration in the serum of healthy subjects reported by *Minoia and co-workers* (12), obtained in Lombardia, Italy, from population of 916 subjects were 6 ± 0.36 (mean ± standard deviation) with the range of 1–10.9 μg/L. Our study obtained a similar range of normal values, that is, <0.9–12 μg/L, although the included population was much smaller.

Aluminium intoxication incidents in dialysis centres were already reported in various countries like USA (13), Venezuela (14) Belgium (15) and in some individual case studies of patients dialysed at home in Great Britain (16–18). High aluminium in dialysis fluids and in serum of patients was found for two main reasons: inadequate water treatment procedure or some installation failure. These experiences point out the need for frequent water and fluid monitoring in all dialysis units. The present study shows linear relation between water and serum aluminium concentration with the highest serum aluminium mean value of about 140 μg/L. At very low concentrations of aluminium in treated water, the mean serum aluminium concentration in patients remains at 19 μg/L with a wide range of 0.9–79 μg/L, though not statistically different from the normal value. *Douthat and co-workers* (19) found that even a change in aluminium from 1.1 to 3.2 μg/L in dialysis fluids sufficed to induce incre-

ments in serum aluminium levels. This indicates that the dialysis procedure accumulates aluminium in patients to a certain degree even at very low and aluminium controlled water concentrations. The same was observed in the present study, since some serum aluminium values were higher than 60 μg/L in the dialysis centre with adequately controlled water. This concentration was suggested as the threshold level for desferrioxamine test according to the Consensus conference held in 1993 (20). Therefore, the regular monitoring of aluminium should not be restricted to treated water and dialysis fluids, but should include the serum of patients on dialysis.

Acknowledgements The authors are grateful to Professor K. Kostial and Dr. M. Piasek for their help and encouragement during this work, to Mrs. Đ. Breški and Mrs. M. Milas for skilful technical contribution and to medical staff in both dialysis centres.

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Sažetak

ALUMINIJ U VODI ZA PRIPRAVU DIJALIZATA I U SERUMU DIJALIZIRANIH PACIJENATA

Svrha ovog rada bila je: a) razviti i verificirati metodu određivanja aluminija (Al) u serumu elektrotermalnom atomskom apsorpcijskom spektrometrijom (ET AAS), b) odrediti koncentraciju aluminija u serumu zdravih ljudi i c) usporediti koncentraciju aluminija u vodi za pripravu dijalizata i u serumu pacijenata na dijalizi.

Metoda ET AAS sastoji se u razrjeđivanju uzorka na koncentraciju mjernog područja od 2 do 10 µg Al/L i primjenu modifikatora matriksa $Mg(NO_3)_2$. Mjerenjem referentnih uzoraka dobiveno je iskorištenje od 98 i 105% za Seronorm odn. BRP otopinu albumina. Ponovljivost metode (koeficijent varijabilnosti izražen kao %) unutar dana je 5,6 odn. 9% i između dana je 3,5 odn. 8,6%. Granica detekcije metode je 0,9 µg Al/L, a karakteristična masa je 3,4 pg Al. U skupini od 50 zdravih ispitanika dobivena je vrijednost aluminija u serumu od 4,5 ± 2,8 (aritmetička sredina ± standardna devijacija) s rasponom od 0,9 do 12 µg/L. Koncentracije aluminija u deioniziranoj vodi za pripremu dijalizata i u serumu pacijenata na dijalizi mjerene su u dva različita centra za dijalizu (centar A i B) u Hrvatskoj 1995. do 1997. godine. Nakon kvara na uređaju u centru A voda za pripremu dijalizata sadržavala je 125 µg Al/L, a koncentracija Al u serumu pacijenata u to vrijeme iznosila je 140 + 54 (N=9). Nakon servisnog popravka vrijednosti Al u vodi snižene su na 8 odn. 27 µg Al/L, a u serumu pacijenata na 30 ± 30 (N=50) odn. 75 ± 38 (N=50). U centru B voda za pripravu dijalizata bila je tijekom praćenja ispod granice detekcije metode, tj. <0,9 µg Al/L. U isto vrijeme serumi pacijenata iznosili su 19 ± 22 (N=21) odn. 19 ± 11 (N=16) µg Al/L.

Rezultati pokazuju linearnu povezanost između koncentracije aluminija u vodi za pripravu dijalizata i koncentracije aluminija u serumu pacijenata na dijalizi. Mali porast koncentracije aluminija u vodi odražava se na porastu aluminija u serumu. Stoga bi redovita kontrola aluminija ne samo u vodi za pripravu dijalizata već i u serumu pacijenata bila nužna.

Ključne riječi:
aluminij u serumu, dijaliza, elektrotermalna atomska apsorpcijska spektrometrija, određivanje aluminija

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