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PREDICTIVE VALUE OF SERUM ALUMINIUM LEVELS FOR BONE ACCUMULATION IN HAEMODIALYZED PATIENTS

S. COSTANTINI *(a)*, R. GIORDANO *(a)*, I. VERNILLO *(a)*, A. PICCIONI *(b)* and G.A. ZAPPONI *(b)*

(a) Laboratorio di Tossicologia Applicata; (b) Laboratorio di Igiene Ambientale, Istituto Superiore di Sanità, Rome, Italy

Summary - *Uremic patients undergoing long-term dialysis risk accumulating tissue aluminium burdens and developing aluminium-related syndromes, such as dialysis encephalopathy and osteomalacia. A statistical retrospective study on 253 uremic subjects was carried out to verify the predictive value of serum aluminium levels on bone aluminium accumulation. Serum and bone samples collected at the same time were analyzed for aluminium content. Analyses were performed by graphite furnace atomic absorption technique. The results verified bone aluminium concentrations of ≤ 60 mg/kg d.w. (dry weight) in 144 patients and greater concentrations in 109 patients. The statistical discriminant analysis showed that serum levels can be predictive in aluminium bone accumulation (lower or greater than 60 mg/kg) with about a 7% margin of error. This value may be further reduced to about 2% if two threshold limits are used (53-81 µg/l). The specificity and sensitivity of the test were 89.6% and 83.5%, respectively.*

Riassunto (Predittività dei livelli serici di alluminio sull'accumulo osseo nei pazienti dializzati) - *Pazienti uremici dializzati a lungo termine sono soggetti a rischio di accumulo tessutale di alluminio e possono sviluppare alcune sindromi alluminio-associate, quali encefalopatia ed osteomalacia. Al fine di verificare il valore predittivo della alluminemia per l'accumulo di alluminio nel tessuto osseo, è stato condotto uno studio statistico retrospettivo su 253 pazienti uremici in dialisi. I campioni di siero e di tessuto osseo sono stati analizzati mediante spettroscopia di assorbimento atomico con fornace di grafite. I risultati hanno mostrato che 144 soggetti avevano una concentrazione di alluminio nel tessuto osseo ≤ 60 mg/kg (peso secco), mentre 109 presentavano livelli più alti. L'analisi statistica discriminante ha evidenziato che i livelli di alluminio nel siero possono essere predittivi per l'accumulo nel tessuto osseo (inteso come minore o maggiore di 60 mg/kg) con un errore di circa il 7%. Se due limiti-soglia sono considerati, ad esempio 53-81 µg/l, l'errore può essere ulteriormente ridotto al 2%. I valori della specificità e della sensibilità del test sono risultati rispettivamente 89,6% e 83,5%.*

Introduction

Aluminium is the third most abundant element in the earth's crust and it is widely diffused. However, until the 1970's, the biological interest in this element was relatively low, its toxic effects being limited to industrial exposure of aluminium dust [1].

It is now well known that aluminium accumulation plays an important role in the pathogenesis of several disorders of uremic patients undergoing long-term dialysis treatment. These clinical disorders include encephalopathy, osteomalacia dialysis osteodystrophy, extraskeletal calcification, and anaemia [2, 3]. For these patients, both inadequate treatment of tap water and aluminium contamination of salts used for the preparation of the dialysis bath have been recognized as potential sources of element exposure [4]. Furthermore, another cause of accumulation is the oral ingestion of aluminium compounds administered as phosphate binding agents to prevent excess gastrointestinal absorption of phosphate. In fact, the aluminium in these compounds is absorbed by the intestine, though only to a small extent [5]. Although there is still much to be understood about aluminium toxicity (e.g. mechanism, toxic concentration, factors governing metabolism), the existing knowledge is such as to make aluminium an important health hazard for renal dialysis patients.

The European Economic Community, aware of this problem, adopted a resolution in June 1986 [6] aimed at minimizing dialysis patients' exposure to aluminium. The EEC also recommended the maximum acceptable aluminium levels for all solutions and waters used in dialysis treatment suggesting that serum aluminium should be monitored quarterly. Thus, aluminium levels of 60 µg/l or more are considered an indicator of excessive build-up of the Al body burden. Concentrations above 100 µg/l are an indicator of the need for a more frequent monitoring and surveillance. Finally, aluminium concentrations above 200 µg/l should never be exceeded.

Although some studies indicate that serum aluminium levels do not provide a reliable index of the total amount deposited in tissues [7, 8], serum monitoring certainly

represents the first parameter for a diagnostic approach to aluminium exposure. Aluminium accumulation in bone, however, causes a vitamin-D-refractory renal osteodistrophy, typical symptoms being bone pain and muscle weakness.

To evaluate the clinical usefulness of this parameter to predict bone aluminium accumulation, a retrospective study of serum aluminium and quantitative bone aluminium contents was performed on 253 uremic patients undergoing regular haemodialysis treatment.

Patients and methods

Between 1983 and 1987 serum and bone specimens of patients clinically suspected of possible aluminium intoxication were analyzed for aluminium content. The main characteristics of the population studied were the following:

uremic patients: 253, males 132 (52.2%), females 121 (47.8%);

hospital haemodialysis: 100%;

dialysis age (years): 7.2 ± 4.1, males 7.1 ± 4.0, females 7.3 ± 4.3;

aluminium hydroxide therapy: 100%;

tap water treatment (deionization): 100%.

Serum and bone (transiliac biopsy) samples of each patient were collected at the same time. To avoid aluminium contamination, all the necessary precautions were adopted in specimen collections.

Chemical analysis

Analyses of aluminium in serum and bone tissue were carried out by graphite furnace atomic absorption technique (Perkin-Elmer model 430 atomic absorption spectrophotometer, equipped with a HGA-500 graphite furnace and an AS-40 autosampler, Perkin-Elmer model 5100 Zeeman atomic absorption spectrophotometer equipped with a HGA-600 graphite furnace and an AS-60 autosampler) using methods previously described [9, 10]. Briefly, serum samples were analyzed, after dilution with doubly-distilled water, in a standard graphite ZrC-coated tube. Fat was removed from bone samples with a chloroform and methanol mixture (1+1); bones were washed in doubly-distilled water and dry-ashed at 550 °C overnight. Good recovery (98.9 ± 3.2%) with Al standard both in tricalcium phosphate and in bone matrix permitted the use of this temperature. Dry ashing was repeated, alternating the treatment with 250-500 µl of nitric acid (Suprapur, Merck, Germany) until white ash occured. Residues were dissolved in nitric acid and made up to suitable volume. Determinations were performed by using a graphite pyrolitic tube with L'Vov platform, modifying the matrix with magnesium nitrate. All quantifications were effected by the standard addition method.

Statistical analysis

The linear regression test was applied to calculate the correlation between aluminium concentration values in bone tissue and serum. The discriminant analysis was used to establish the predictive value of serum aluminium levels in bone accumulation. Since the original data did not show a normal distribution and hence did not permit the use of parametric tests, the values have been transformed into their natural logarithms. Considering the limits proposed by the Resolution of the European Economic Community for the aluminium levels in plasma or serum, the value of 60 µg/l indicating an excessive build-up of aluminium body burden, was chosen as a "limit of concentration" in the discriminant analysis.

Results

The correlation plot between aluminium concentration values in bone tissue and serum is reported in Fig. 1. The test evidenced a highly significant correlation ($p < 0.001$) with the following equation:

$$Y = 0.960\ X + 0.469 \qquad r = 0.785$$

Fig. 1 also shows the confidence limits (95%) of the data used in the correlation. Results of aluminium concentration both in bone and serum are reported in Table 1.

The more frequent serum aluminium values (Als) fall in the 31-60 µg/l range (33.6%), whereas the bone aluminium (Alb) ones are in the 31-60 mg/kg d.w. (dry weight) range (37.5%). The equation presents a value of 62 mg/kg of bone aluminium when a value of 60 µg/l of serum aluminium is considered. Bone aluminium distributions as a function of serum concentrations (lower or greater than 60 µg/l, respectively) are reported in Fig. 2.

The results of the discriminant analysis show that 144 (56.9%) patients (group 1) had Alb ≤ 60 mg/kg d.w. (X = 35 ± 15) whereas 109 (43.1%) patients (group 2) had Alb > 60 mg/kg d.w. (X = 115 ± 52). In group 1, only 129

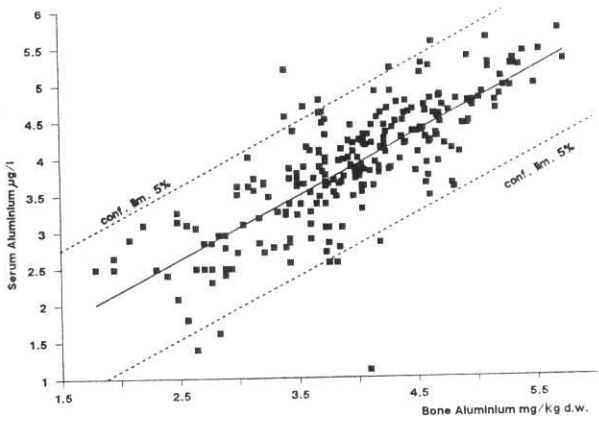

Fig. 1. - Bone serum aluminium. Regression line (log-data). Dotted lines represent the prediction interval for individual value of Y.

Table 1. - *Distribution of aluminium in serum and bone tissue (number of patients = 253)*

Serum

$\bar{X}$ = 66.1 ± 52.6 µg/l
Range = 4-304 µg/l

Class (µg/l)	Number of patients	Frequency (%)	Mean ± SD (µg/l)
≤ 10	6	2.4	5.2 ± 1.8
11-30	56	22.2	18.1 ± 5.6
31-60	85	33.6	44.8 ± 7.8
61-100	55	21.7	77.7 ± 11.4
101-200	44	17.4	130 ± 36
201-304	7	2.8	244 ± 36

Bone

$\bar{X}$ = 69.2 ± 52.7 mg/kg d.w.
Range = 6-313 mg/kg d.w.

Class (mg/kg)	Number of patients	Frequency (%)	Mean ± SD (mg/kg)
≤ 10	5	2.0	7.4 ± 1.1
11-30	46	18.2	19.4 ± 5.5
31-60	95	37.5	43.4 ± 12.1
61-100	53	21.0	74.5 ± 18.2
101-200	45	17.8	126 ± 24
201-313	9	3.6	241 ± 44

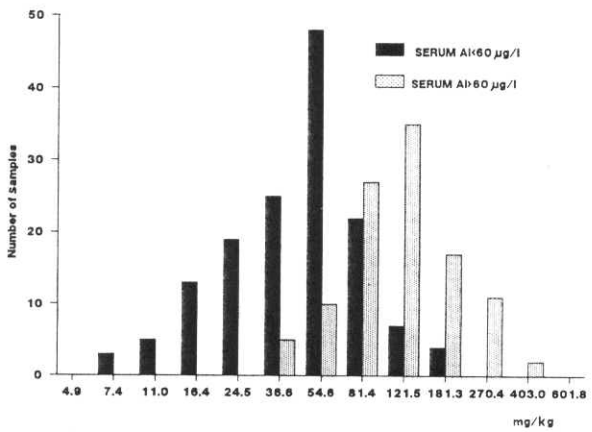

Fig. 2. - Bone aluminium distributions when serum Al concentration is lower than 60 µg/l or greater than 60 µg/l.

subjects with Als ≤ 60 µg/l (X = 30 ± 15) were correctly identified by serum values while 15 patients (10.4%) were false positives showing Als > 60 µg/l (X = 81 ± 31). In group 2, only 91 subjects with Als > 60 µg/l (X = 119 ± 53) were correctly identified by serum values while 18 patients (16.5%) were false negatives showing Als ≤ 60 µg/l (X = 44 ± 8). The specificity and sensitivity of the test were 89.6% and 83.5%, respectively.

On the basis of these results, the probability of having Alb ≤ 60 mg/kg when Als is ≤ 60 µg/l or Alb > 60 mg/kg when Als is > 60 µg/l, is of 93%. If a classification threshold of 60 µg/l is assumed, the classification error expected is of about 7%. Instead, if two threshold limits are used and an "uncertainty interval" is assumed, the classification error may be reduced to a lower level. For instance, if we assume 56-75 µg/l as a range of uncertain classification, the classification error is of about 3%. This value may be further reduced to about 2% if the 53-81 µg/l range is considered.

The equation calculated for the correlations found between the dialysis age and the aluminium concentrations in serum and in bone are the following:

dialysis age / serum aluminium

$Y = 0.45 X + 3.18$

$r = 0.448$

$p < 0.001$

dialysis age / bone aluminium

$Y = 0.47 X + 3.21$

$r = 0.516$

$p < 0.001$.

Discussion

The results reported in Table 1 indicate that 147 out of 253 (58.2%) subjects studied have serum aluminium concentrations lower than 60 µg/l, with a mean value of 33 ± 17 µg/l. Since the European Economic Community considers that an increased aluminium intake occurs above 60 µg/l, and that some authors [11] consider it unlikely that aluminium-related bone disease is present when the serum aluminium concentration is below 50 µg/l, results evidence a rather safe picture for more than 50% of the examined

population. In addition, 62 out of 147 (42.2%) subjects have aluminaemia in a very low concentration range (below 30 µg/l). The following considerations should be made about the group of patients with a serum aluminium concentration greater than 60 µg/l (X = 112 ± 51): 55 out of 253 (21.7%) subjects had aluminium concentrations in a 60-100 µg/l range requiring a careful surveillance of the patients; 44 out of 253 (17.4%) were in a risk range (100-200 µg/l), sometimes considered pathological with possible aluminium-related bone disease [11-14]. Nevertheless, only 7 out of 253 (2.8%) subjects have an aluminium concentration above 200 µg/l, a value often associated with osteomalacia [11, 12].

Generally, serum levels found in this study agree with those obtained by other Italian authors [15] who carried out an epidemiological study on 1159 patients, including all the dialysis centers of the Veneto region. The study was performed on serum, waters and dialysis fluids.

Even though the role of aluminium in affecting (directly or indirectly) bone formation and/or mineralization [16] is controversial, it is however evident that aluminium accumulation in bone is associated with abnormalities in cell function and bone structure [17]. Moreover, an established aluminium intoxication is difficult to treat and a successful management of the patient is likelier when intoxication is early. Consequently, an early recognition of aluminium overload is of great importance.

Our results indicate that the mean Als value of the group with Alb ≤ 60 mg/kg (Als = 35 ± 15 µg/l) is statistically lower ($p < 0.001$) than the one found in the group with Alb > 60 mg/kg (Als = 119 ± 53). This finding offers the possibility to discriminate between patients with and those without bone aluminium deposits. However, a certain overlapping occurs between the two distributions (Fig. 2) determining intermediate values of specificity (89.6%) and sensitivity (83.5%). In fact, these values fall in the 65 to > 90% range, the former considered a statistically poor value, whereas the latter is considered a good value. On the other hand, it is inevitable that a recent exposure to aluminium causes the presence of "false positive" elements and a fairly low serum aluminium concentration at the time of bone biopsy (with high Al content) could reflect a recent reduction of the exposure.

In general, aluminium serum levels can be considered a reliable means of detecting recent exposure; moreover, we feel that it is also a fair indicator of bone burden.

Unfortunately, both financial and analytical implications render the current monitoring of aluminium in serum, waters or dialysis fluids an objective not yet reached by many Italian dialysis centers. Aluminium analysis, compared to other clinical analyses, is fairly expensive both in terms of equipment and running costs. Considerable expertise is also required.

Nevertheless, because of the great importance of dialysis treatment, efforts must be made to set up regional analysis centers capable of performing regular monitoring.

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