The enigma of aluminum deposition in bone tissue from a patient with chronic kidney disease: a case report

O enigma da deposição de alumínio no tecido ósseo de um paciente com doença renal crônica: relato de caso

Abstract

About four decades ago, the relationship between dialysis-dementia and aluminum (Al) began to be established. The restriction of drugs containing Al and improvements on water quality used for dialysis resulted in the clinical disappearance of Al intoxication. However, high prevalence of Al deposition in bone tissue from Brazilian dialysis patients is still being detected. Through the case report of a patient on hemodialysis (HD) for one year, presenting significant Al deposition in bone tissue, we speculated if this problem is not being underestimated. We used extensive investigation to identify potential sources of Al exposure with a careful review of medication history and water quality controls. Al concentration was measured by different methods, including mass spectrometry, in poly-electrolyte concentrate solutions and solution for peritoneal dialysis, in an attempt to elucidate the possible sources of contamination. The objective of this case report is to alert the medical community about a potential high prevalence of Al deposition in bone tissue and to discuss the possible sources of contamination in patients with chronic kidney disease (CKD).

Keywords: Kidney Failure, Chronic; Dialysis; Bone Diseases, Metabolic; Aluminum.

Introduction

Aluminum (Al) is the most abundant metal on earth and human beings are often exposed to it.1 The accumulation and toxicity of this metal was noted in hemodialysis (HD) patients in the 1970’s, and osteomalacia, anemia, and dementia were associated with exposure to water, dialysate preparations, or drugs containing Al.2-4 Since improvements on water treatment were established and the use of non-Al-containing phosphate (P) binders became standard practice, the prevalence of Al intoxication with clinical signs almost disappeared.4-5 Therefore, it was assumed...
that Al-related bone diseases would also have disappeared. This potential misconception was supported by clinical and serum Al levels evaluations only, instead of the gold standard method: bone biopsy stained by solochrome azurine.

Brazil is one of the countries with the largest number of dialysis patients in the world and has about 700 dialysis units. Most units use reverse osmosis for water treatment, and quality requirements are similar to the European and American guidelines, being controlled under Federal legislation. Four laboratories in Brazil are specialized on renal osteodystrophy and perform bone histomorphometric analysis and histological studies for Al detection. These centers have an accumulated experience of more than 5,000 bone biopsies from chronic kidney disease (CKD) patients. Recently, the Brazilian Registry of Bone Biopsy (REBRABO) was created as a research platform on this field. Data analysis has detected a high prevalence of Al deposition in bone samples from Brazilian CKD patients over the decades. Therefore, we claim attention to potential under-diagnosis of Al deposition in bone tissue in other countries as well.

We present the case of a patient who had been on HD for just one year and was diagnosed with Al deposition in bone tissue. An extensive investigation was carried out to identify potential sources of Al exposure.

**Case Report**

A 36-year-old man with CKD of undetermined etiology started peritoneal dialysis (PD). After 3 years, he switched to HD due to an episode of fungal peritonitis. He remained clinically stable during the first year of HD and never presented any signs or symptoms related to mineral and bone metabolism disorders, such as bone pain, pruritus, muscular weakness, pathological fracture, signs of vascular calcification or neurological symptoms. His physical examination was normal. Overtime he developed asymptomatic hyperparathyroidism, presenting serum intact parathyroid (iPTH) levels of 467 pg/mL, P of 3.8 mg/dL, calcium (Ca) of 9.5 mg/dL, alkaline phosphatase (AP) of 92 IU/L, and Al of 13 mcg/L [methodology: graphite furnace-atomic absorption spectrometry (GFAAS); reference range: < 30 mcg/L]. At this moment, the patient was included in a clinical study, and a transiliac bone biopsy was performed. The sample obtained consisted of two cortical and trabecular bone samples revealing the diagnosis of osteitis fibrosa. Unexpectedly, the coloration of solochrome azurine was positive for Al, covering 50% of the bone surface. Pearls’ staining was positive for iron in a similar extent (Figure 1A to 1D). Treatment with desferoxamine at 5 mg/kg once a week for 6 months was initiated, with follow-up exams revealing serum levels of Ca 10.2 mg/dL, P 2.2 mg/dL, iPTH 263 pg/mL, AP 47 IU/mL, and Al 4.7 mcg/L. At the end of the treatment, the patient was still asymptomatic and without signs of Al intoxication or bone disease. One year after being submitted to bone biopsy the patient underwent renal transplantation.

The unexpected diagnosis of Al deposition has led to the investigation of sources of exposure, such as medications, water for HD, polyelectrolyte concentrates, and PD solution bags. Review of medical records has shown the patient had never used antacids, Al-based P binders, or any medications that could deliberately contain Al. In the last 3 years, he had never presented alterations in annual serum Al levels (GFAAS, reference range: < 30 mcg/L). Al detection analyses in HD water treated by reverse osmosis provided negative results (two samples, separated by one year) (methodology: inductively-coupled plasma optical emission spectrometry; reference range < 10 μg/L).

We tested bone tissue samples, water used in the dialysis unit, polyelectrolyte concentrate solutions, and PD solution bags using inductively-coupled plasma mass spectrometry (ICP-MS) with laser ablation (LA) techniques. The chemical elements present in the sample were ionized by high plasma temperature. Only ions Fe⁺ and Al⁺ were selected, generating a signal proportional to their quantities in the samples. The technique is based on the use of a laser for ablating the sample, and the vapor generated in the process is transported by an inert gas (argon) to the inductively coupled plasma torch. LA-ICP-MS lecture can be converted to an imaging mode containing the distribution of metal in the tissue. This qualitative analysis was performed on bone tissue using the LA-ICP-MS technique, through a Perkin-Elmer brand equipment (DRC-e model) and a LA unit (New
Figure 1. Representative images of bone tissue. (A) Thin cortical and trabecular bone with an increased trabecular separation (x40 magnification). (B) Trabecular bone with osteoid (x400). (C) Solochrome azurine staining revealing in blue (black arrows) the aluminum deposition in bone interface (mineralization front) along almost the entire trabeculae (x400); (D) Pearls staining revealing deposits of iron predominantly in trabecular bone. *bone marrow; black arrows: cortical bone; white arrows: trabecular bone; black broad arrow, osteoid; arrows: black, Al deposition in Figure 1C and iron deposition in Figure 1D. Images of bone tissue showing the deposition of Al and Fe, constructed by LA-iMageS software with data obtained from the analysis of LA–ICP-MS. (E) Distribution of Al predominantly in trabecular bone tissue; (F) Distribution of Fe predominantly in bone marrow. Sidebars refer to the intensity of the elements present in the tissue: high intensity (dark red and red) or low intensity (dark blue and blue), in-between: average intensity.
Table 1: Quantification of Aluminum by ICP-MS in Different Water Samples and Solutions Used in Dialysis Unit. The Concentration of Al in All Samples Was Very Close to the Value of the Normalization Concentration Added to Each Sample

| Samples                                | Al concentration (µg/L) |
|----------------------------------------|-------------------------|
| PCHD (acid) trademark A                | 46.7 ± 0.8              |
| PCHD (acid) trademark B (sample 1)     | 50.2 ± 0.9              |
| PCHD (acid) trademark B (sample 2)     | 50.3 ± 1.5              |
| SCB trademark A (sample 1)             | 46.9 ± 0.5              |
| SCB trademark A (sample 2)             | 47.6 ± 0.5              |
| Peritoneal dialysis solution trademark C | 50.4 ± 0.7            |
| Reverse osmosis outlet water (sample 1)| 51.5 ± 0.6              |
| Reverse osmosis outlet water (sample 2)| 51.5 ± 0.7              |
| Pre-treatment inflow water (sample 1)  | 51.5 ± 1.0              |
| Pre-treatment inflow water (sample 2)  | 49.2 ± 0.5              |
| Dialysate at the input of the HD machine | 49.7 ± 0.5          |

HD: hemodialysis; PCHD: polyelectrolyte concentrate for hemodialysis; SCB: sodium bicarbonate concentrate; Al: aluminum.
- 0.1%) are absorbed from food sources. Factors that may influence absorption and its bioavailability are compounds that bind to Al in the intestinal lumen, gastric acidity, and hardness of water consumed.\textsuperscript{21} Patients with celiac disease may have increased intestinal permeability to Al, and can thus develop Al-related bone disease.\textsuperscript{22} None of these conditions was observed in our patient.

Unfortunately, we did not evaluate Al content in the ingested water and intravenous drugs used by the patient. We believe that the main source of Al exposure for CKD patients is the water used for dialysis, although we could not prove this. The ICP-MS could be a differential and complementary technique for a frequent evaluation of fluids and drugs used in the treatment of these patients, aiming to avoid exposure to Al. Additionally, its complementary technique (LA-ICP-MS) can discriminate safely which metal is deposited in the tissue. In this case report a limited amount of samples was analyzed, while the patient had contact with 360 L or more of water per week for years. We cannot affirm that polyelectrolyte concentrates and PD solution bags were not sources of contamination, since only a few samples were analyzed.

**CONCLUSION**

Al intoxication may be largely under-diagnosed, perhaps in several regions of the world. There is an urgent need for clinical studies with bone biopsy in this field in order to confirm our hypothesis. Considering that doses of Al in fluids have limited diagnostic value and bone biopsy is an invasive procedure and restricted to a few centers, both ICP-MS and LA-ICP-MS are promising techniques that can be used to understand the phenomenon of Al intoxication in patients on dialysis, helping in the identification of contamination sources. Systemic Al intoxication is an unusual event nowadays, but deposition of Al in bone tissue can be a frequent event, which can cause important clinical outcomes, such as fractures and death.

**ACKNOWLEDGMENT**

The authors thank Espaço da Escrita - Coordenadoria Geral da Universidade - UNICAMP - for the language services provided, and Wagner Vasques Dominguez for the technical assistance.

**REFERENCES**

1. Martin BR. Chemistry of Aluminum. In: De Broe M, Coburn JW, eds. Aluminum and renal failure. Dordrecht: Kluwer Academic Publishers; 1990. p. 7-26.
2. Dunea G. Dialysis dementia: an epidemic that came and went. ASAIO J 2001;47:192-4.
3. Mahurkar SD, Salta R, Smith EC, Dhar SK, Meyers L Jr, Dunea G. Dialysis dementia. Lancet 1973;1:1412-5.
4. Sandhu G, Djebali D, Bansal A, Chan G, Smith SD. Serum concentrations of aluminum in hemodialysis patients. Am J Kidney Dis 2011;57:523-5.
5. Malluche HH. Aluminum and bone disease in chronic renal failure. Nephrol Dial Transplant 2002;17:21-4.
6. Oliveira MB, Romão JE Jr, Zatt R. End-stage renal disease in Brazil: epidemiology, prevention, and treatment. Kidney Int Suppl 2005;S82-6.
7. Comission of the European Community (CEC). Resolution 86/C184/04 of the Council concerning the protection of dialysis patients by minimizing the exposure to aluminium. Off J Eur Communities 1986;C184.
8. de Oliveira RB, Barreto FC, Custódio MR, Gueiros JE, Neves CL, Karohl C, et al. Brazilian Registry of Bone Biopsy (REBRA-BO): design, data elements and methodology. Braz J Nephrol 2014;36:352-9.
9. Araújo SM, Ambrosio P, Lobão RR, Coarsi H, Møysès RM, Barreto FC, et al. The renal osteodystrophy pattern in Brazil and Uruguay: an overview. Kidney Int Suppl 2003; 85:S4-6.
10. Carbonara CEM, dos Reis LM, Sampaio EDA, Canziani MEF, Møysès RMA, de Carvalho AB, et al. Relation between type of osteodystrophy renal and manifestations clinicas in patients with DMO – DRC. In: 28th Congresso Brasileiro de Nefrologia; 2016 Sep 14-17; Maceió, AL, Brazil.
11. Taylor A, Walker AW. Measurement of aluminium in clinical samples. Ann Clin Biochem 1992;29:377-89.
12. Buchanan MR, Ihle BU, Dunn CM. Haemodialysis related osteomalacia: a staining method to demonstrate aluminium. J Clin Pathol 1981;34:1352-4.
13. Ellis HA, Pang MM, Mawhinney WH, Skiline AW. Demonstration of aluminium in iliac bone: correlation between aluminium and solochrome azurine staining techniques with data on flameless absorption spectrophotometry. J Clin Pathol 1988;41:1171-5.
14. Fernández-Martín JL, Menéndez P, Acuña G, Canteros A, Gómez C, Cannata JB. Staining of bone aluminium: comparison between aluminium and solochrome azurine and their correlation with bone aluminium content. Nephrol Dial Transplant 1996;11:380-5.
15. Muñoz JJ, Drigo SA, Barros-Filho MC, Marchi FA, Scapulario C, Pessoa GS, et al. Down-Regulation of SLC8A1 as a Putative Apoptosis Evasion Mechanism by Modulation of Calcium Levels in Penile Carcinoma. J Urol 2015;194:245-51.
16. López-Fernández H, de S Pessôa G, Arruda MA, Capelo-Martinez JL, Fdez-Riverola F, Glez-Peia D, et al. LA-tMageS: a software for elemental distribution bioimaging using LA–ICP–MS data. J Cheminform 2016;8:65-75.
17. Rao DS, Shih MS, Mohini R. Effect of serum parathyroid hormone and bone marrow fibrosis on the response to erythropoetin in uremia. N Engl J Med 1993;328:171-5.
18. Díaz-Corte C, Fernández-Martín JL, Barreto S, Gómez C, Fernández-Coto T, Braga S, et al. Effect of aluminium load on parathyroid hormone synthesis. Nephrol Dial Transplant 2001;16:742-5.
19. Felsenfeld AJ, Machado L, Bover J, Trinidad P, Rodrigue M. Effect of aluminium on the development of hyperparathyroidism and bone disease in the azotaemic rat. Nephrol Dial Transplant 1993;8:323-34.
20. Bohrer D, Bertagnoli DC, de Oliveira SM, do Nascimento PC, de Carvalho LM, Pombilim SC. Drugs as a hidden source of aluminium for chronic renal patients. Nephrol Dial Transplant 2007;22:605-11.
21. Driücke TB. Intestinal absorption of aluminium in renal failure. Nephrol Dial Transplant 2002;17:13-6.
22. Chappard B, Bizot P, Mabilleau G, Hubert L. Aluminium and bone: Review of new clinical circumstances associated with Al(3+) deposition in the calcified matrix of bone. Morphologie 2016;100:95-105.