Analytical Problems Encountered in Determining Aluminum Status from Hair in Controls and Hemodialyzed Patients

P. Chappuis,1 L. Duhaux,1 F. Paolacci,1 M. C. de Vernejoul,2 and F. Rousselet1,3

The problems involved in evaluating aluminum concentrations in hair are reviewed, especially those concerning removal of contaminating metals, a critical factor. In the few published studies of Al concentrations in hair, acetone was usually used for its removal. Here, its use in the washing sequence was found to give less precise results and higher Al values than the use of isopropanol. With isopropanol, the whole analysis can be done in a single tube. We compared results with those in the literature. We found that the Al concentration in the hair of control subjects was not related to sex or hair color and that there was a highly significant ($P < 0.001$) difference between values for control subjects and hemodialyzed patients: 126 (SD 58) nmol/g, $n = 49$, vs 226 (SD 104) nmol/g, $n = 39$, respectively.

Additional Keyphrases: variation, source of · isopropanol vs acetone solvent · chronic renal failure · atomic absorption spectrometry

Patients with chronic renal failure who are undergoing hemodialysis are greatly at risk of Al accumulation, which can induce vitamin D-resistant osteomalacia, dialysis encephalopathy, and microcytic hypochromic anemia (1). This accumulation comes chiefly from the dialysate, or intestinal absorption after oral administration of phosphate binders containing Al, or both (2).

Al overload can be assessed by measuring the Al content of transiliac bone, but the sampling procedure is painful. Consequently, a reliable noninvasive technique of Al overload measurement would be extremely useful. For this purpose, hair sampling has many advantages over materials such as blood and bone, because samples are easily and repeatedly available and require no special storage conditions. In addition, the slow growth of hair means it can provide retrospective information about the burden of Al in the body. Unlike blood, short-term variations in hair concentrations of analytes are averaged out, so that the values for Al in hair reflect its accumulation over long periods (3).

So far, few studies of Al concentrations in hair have been reported (4–6) and recent results are conflicting. We undertook this study in an attempt to clarify the situation.

Materials and Methods

Subjects

The participants in the study were 40 hemodialyzed patients, ages 23–75 y (mean 49, SD 12), who had been on dialysis for 5.6 (SD 3.2) y. Most of them were given gels containing Al to maintain the concentration of phosphorus in serum at <1.9 mmol/L.

The controls, 49 healthy volunteers, consisted of 27 women and 22 men (mean age 42, SD 11 y). Their hair was analyzed for Al but none of them was taking Al-containing medication.

Thirty other volunteers provided a pool of hair, which was thoroughly mixed with a polystyrene rod. We used this to assess contamination-removal methods, digestion procedures, and analytical accuracy.

The samples from the patients and controls were then tested by the method chosen.

Procedures

Hair sampling. Samples of suboccipital hair from patients and controls were cut into several pieces with stainless-steel scissors and placed in individual graduated polystyrene tubes. Both groups were asked to complete a questionnaire on the brands of shampoo, dyes, or bleaches used.

Contamination-removal methods. Method 1: Part of the pool of hair from the 30 volunteers was treated according to Ryabukhin (7) by five consecutive 10-min washes: one in acetone, three in de-ionized water, and another in acetone.

Method 2: Another part of the hair pool was washed by shaking it in a mixer for four consecutive baths for 5 min each: isopropanol, water, water again, and isopropanol. This method gave the best results (see Results) and accordingly was applied to the patients' and controls' samples ("recommended procedure").

Digestion procedures. About 60 mg of hair per sample was digested. We eliminated digestion procedures requiring glass, quartz, or even Teflon devices, which pose a potential risk of contamination. We tested two extremely simple methods: digestion in a 75 g/L aqueous solution of tetramethylammonium hydroxide at 90 °C for 2 h (5) and overnight digestion in pure concentrated nitric acid at 60 °C.

Hair sample treatment and recommended procedure. Remove contamination by method 2 and dry the samples overnight. Weigh the samples (approximate weight: 60 mg) and digest them for 12 h at 60 °C in 2 mL of "Ultrapur" nitric acid ($d = 1.40$; Prolabo, Paris, France). Dilute the samples with de-ionized water in the same graduated polystyrene tube to a total volume of 20 mL and use 10 μL of this solution for measurement by graphite furnace atomic absorption spectrometry. We made measurements in triplicate, with the standards addition technique (i.e., addition of 0, 0.5, 1, or 1.5 μmol of Al per liter), and a Philips SP 2900 spectrometer (wavelength, 309.3 nm; drying temperature, 90 °C for 35 s; ashing temperature, 1400 °C for 35 s; and atomizing temperature, 2700 °C for 5 s). The atomizer was an uncoated standard graphite tube.

All laboratory ware used—such as plastic tubes and pipette tips—was previously checked for Al contamination. Polypropylene cups for the autosampler were stored in dilute (100 g/L) nitric acid, rinsed with de-ionized water, and dried before use in analysis.

Statistical analysis. Because the distributions were not gaussian, we used the Mann–Whitney U test for all comparisons.

1 Laboratoire de Biochimie, Hôpital Lariboisière, 75010, Paris, France.
2 INSERM U 18, Hôpital Lariboisière, 75010, Paris, France.
3 Address correspondence to this author, at: Laboratoire de Biochimie Appliquée, Faculté de Pharmacie de Paris V, 4, avenue de l'Observatoire, 75006, Paris, France.

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Results

Analytical Variables

Contamination removal methods. Using our recommended procedure, we found that method 1 yielded a mean of 185 nmol/L (SD 22 nmol/L, CV 13.3%) and method 2 a mean of 132 nmol/L (SD 9 nmol/L, CV 7.2%). Each mean was based on 30 measurements.

Digestion procedure. Digestion in tetramethylammonium hydroxide was not chosen because some hair samples were incompletely digested, even after 3 or 4 h at 90 °C.

Precision. By our recommended procedure, between-run CVs (10 runs) were 4.1% and 3.2%, respectively, for hair Al concentrations of 160 and 130 nmol L. The day-to-day CVs for the same samples (10 runs) were 5.3% and 4.7%. Detection-limit studies showed that 0.6 pmol (volume injected: 10 μL) yielded a 1% absorption signal. This amount represents a detectable Al concentration of 24 nmol/g of dry weight, corresponding to 20 mL of digest.

Accuracy. We tested the accuracy of our method by adding different amounts of aluminum nitrate solution to eight washed hair samples and assaying. Analytical recovery ranged from 84 to 105% (Table 1).

Values Obtained for the Subjects

Healthy controls. Several reports in the literature suggested that differences in the zinc and copper content of human hair are sex-related (8) and also related to melanin concentration in hair (9). We therefore attempted to establish whether or not this also applied to Al. There was no significant difference in Al content, nanomoles per gram of hair, for men and women (123, SD 9, nmol/g, n = 15, vs 127, SD 75, nmol/g, n = 34); nor did we find any relation between hair color and Al content:

- blond hair: 135 (SD 61) nmol/g (n = 9)
- light brown hair: 128 (SD 45) nmol/g (n = 24)
- dark brown hair: 112 (SD 70) nmol/g (n = 8)
- white and gray hair: 123 (SD 65) nmol/g (n = 8).

Hemodialyzed patients. As shown in Figure 1, there was a highly significant difference (P < 0.001) between the mean Al content in hair from control subjects and from hemodialyzed patients (126, SD 58, nmol/g, n = 49, vs 226, SD 104, nmol/g, n = 39).

Discussion

Washing and digestion procedures. Because Al constitutes 1 or 2% of atmospheric dust, surface contamination of hair must be removed by appropriate procedures. This external contamination can originate from the water used for washing hair and from cosmetic or medical treatment, or both. The question arises of whether there is so much of this exogenous Al incorporated into the hair (10) that attempts to remove it (e.g., by the procedure used here) could be ineffective. Indeed, in our study (Figure 1), of the 49 healthy volunteers had high Al values, >200 nmol/g of hair, even though none of them had used any special cosmetic hair treatment or shampoo containing Al. Conversely, more-aggressive agents such as EDTA might also bind endogenous Al from hair (11), thus understimating its presence. The most frequently used washing procedures for Al removal are alternate baths of organic solvents (e.g., acetone) and distilled water (4, 5, 12). Our method, using isopropanol instead of acetone, gave more precise results, probably because the washing and digestion procedures as well as the final dilution were all done in the same polystyrene tube, which is more practical than the glass or quartz tubes used in the other method tested. Moreover, provided the hair snippets cut before the washing procedure are sufficiently short (the ones we used were not more than 0.5 cm long), the easiest and least contaminating method of sample digestion is to add nitric acid directly to the same tube. This avoids transferring the sample elsewhere, which incurs a risk of Al contamination.

Precision and accuracy studies. The precision of our results was comparable to those of Stevens (5) and analytical recovery was satisfactory. The comparison of mean hair Al concentrations in Table 2 shows great differences among them, probably more because of the washing procedure than the analytical technique used. Our values are lower than those reported by most other investigators, especially Stevens (5) and Hewitt and Day (13), who, like ourselves, used atomic absorption spectrometry.

Ryan et al. (14) used ultrasonic treatment in distilled water as a washing procedure. According to them, ethanol and distilled water increased the efficiency of the washing treatment by 14%. This means their median Al value would be 150 nmol/g, which fits our results better than the values of Stevens (5) and Hewitt and Day (13). Moreover, our comparison between the results of two washing procedures (acetone/water/acetone and isopropanol/water/isopropanol) confirms that the use of alcohol in the washing sequence leads to lower Al values than the use of acetone. Salmela et al. (15), who compared different washing procedures, also showed that acetone removed less of the trace element content from hair pools than any other agent. We believe that, although acetone has been recommended and widely used for analysis of Al in hair (7, 12, 13), it might not be entirely suitable for this because, as we found, the acetone washing sequence in the present investigation led to poorer precision. Although the lower Al values obtained with the isopropanol sequence are probably attributable to external contamination removal, we still do not know whether these lower results result from removal of any endogenous Al (10, 11, 14, 16).

![Fig. 1. Distribution of aluminum concentrations in hair of controls (open bars, n = 49) and hemodialyzed patients (shaded bars, n = 39)](image)

Table 1. Analytical Recovery of Aluminum

<table>
<thead>
<tr>
<th>Amount present</th>
<th>Amount added</th>
<th>Amount measured</th>
<th>Recovery, %</th>
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<tr>
<td>19</td>
<td>205</td>
<td>235</td>
<td>105</td>
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<td>71</td>
<td>190</td>
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<tr>
<td>171</td>
<td>390</td>
<td>567</td>
<td>102</td>
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*(Al measured – Al present)/Al added) × 100.
However, this might only apply to Al, in that Boe (17) suggested that no uniform washing procedure can be used for all metals because the sorption behavior and distribution of each metal over a cross-section of the hair shaft varies with the trace element analyzed. Similarly, this author concluded from measurements by particle-induced x-ray emission that there may be differences between the amounts of metal present at the root end and in the distal segments of the same hair fiber, and between the amounts in hair from different parts of the head. This was already noted by Alder et al. (18), who showed that Al concentrations increased distally along head hair. This suggests that external sources (e.g., environmental exposure and cosmetic treatment) could explain this longitudinal variation and might also account for the variability of the results reported here in Table 2, because the exact lengths of the shafts cut were not measured.

Hair aluminum and hair color. Most of the studies mentioning differences in the mineral content of hair of different colors do not consider Al. We found that Al did not vary with hair color, because Al has no known role in melanin synthesis. However, the method of classifying hair color raised the problem of intermediate shades, which are difficult to assign to different categories.

Hair aluminum in renal patients. Marumo et al. (12) reported higher Al values in the hair of patients with chronic renal failure (both nondialyzed and dialyzed) than in controls. According to Winterberg et al. (19), the amount of Al in hair from patients on long-term hemodialysis is "at the upper normal limit." However, Hewitt and Day (13) were unable to distinguish renal patients from controls by analyzing hair for Al.

In addition, as Table 2 shows, the mean Al values in both patients and controls are also highly discrepant.

Our results disagree with those of Hewitt and Day (13), but the number of patients and controls studied by these authors was perhaps too small (13 and 12, respectively) to indicate any difference between the two groups. Here, the difference we found between the Al concentrations in the hair of the controls and hemodialyzed patients (P <0.001) is real in terms of means comparison and reflects prolonged exposure of the renal patients to Al. Nevertheless, because of the overlap between the patient and control groups, we conclude that the Al values in hair of individual patients do not allow prediction of an Al overload and cannot reflect other body stores of Al.

References