Increased serum strontium levels in dialysis patients: An epidemiological survey

IRIS SCHROOTEN, MONIQUE M. ELSEVIERS, LUDWIG V. LAMBERTS, MARC E. DE BROE, and PATRICK C. D'HAESE

Department of Nephrology-Hypertension, University of Antwerp, Antwerp, Belgium

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Background. We previously reported on increased bone strontium levels in dialysis patients with osteomalacia versus those presenting other types of renal osteodystrophy. A causal role of strontium in the development of osteomalacia was established in a chronic renal failure rat model.

Methods. To further elucidate the latter issue and to find out whether dialysis patients from particular centers/countries are at an increased risk for strontium accumulation, a worldwide multicenter study was established. In total, 834 patients from 34 dialysis centers in 23 countries were included. In each of the patients, a serum sample was taken for strontium determination, and water and dialysate samples were taken at the various steps of the water purification process. For each patient clinical data and for each center dialysis modalities were recorded.

Results. Strontium levels in serum of dialysis patients showed major differences between the various centers, ranging from mean values of 25 ± 8 μg/liter in the center with the lowest level up to 466 ± 90 μg/liter in the center with the highest concentration. It is of interest that these high levels were mainly found in developing countries. Furthermore, our data point toward a role of the final dialysate in the accumulation of the element, as indicated by the strong correlation ($r = 0.74$, $P < 0.001$) between mean serum and dialysate strontium levels. As the high tap water concentration of strontium was adequately reduced during the water purification process, contamination of the final dialysis fluid occurred by the addition of concentrates contaminated with strontium. Besides the dialysate, other factors, such as duration of dialysis, vitamin D supplements, or types of phosphate binders, played a less important role in the accumulation of the element.

Conclusions. Data of this multicenter study indicate patients of particular dialysis centers to be at an increased risk for strontium accumulation, the clinical consequence of which is under current investigation.

We recently determined various trace element concentrations in 100 bone biopsies of dialysis patients presenting with various types of renal osteodystrophy. These bone biopsies had previously been taken in the context of studies of the noninvasive diagnosis of adynamic and aluminum (Al)-related bone disease [1, 2]. Histological/chemical examination of the 100 bone biopsies revealed bone strontium (Sr) levels, as well as Sr/calcium (Ca) ratios to be increased in osteomalacic patients. Bone Sr levels were not only elevated compared to individuals with a normal bone histology, but also in comparison to all other types of renal osteodystrophy. Bone Sr and bone Ca levels correlated with each other. The slope of the linear regression curve correlating these parameters was much steeper in the osteomalacic group as compared to the other types of renal osteodystrophy. Furthermore, we found bone Sr levels to correlate with bone Al levels in the group of patients with osteomalacia, suggesting that the development of this disease could be the result of a multi-element effect [3]. A causal role of Sr in the development of osteomalacia was established in an experimental study. Here, oral administration of Sr to a chronic renal failure rat model resulted in the development of distinct osteomalacic lesions. Of interest was that in this study, rats loaded with Al developed adynamic bone, whereas in the group of animals loaded with both Sr and Al, some rats developed adynamic bone, whereas in others, a more severe osteomalacia occurred [4].

Notwithstanding its abundance in nature, little is known about the metabolism of Sr and whether or not it is essential for humans. Sr is a natural constituent of food and beverages. Meat, poultry, vegetables, and fruit contain relatively low amounts of the element, but in cereals, grains, and seafood, the element may be present at concentrations up to 25 mg/kg. Hence, the Sr content of the human diet and the daily intake of the element varies according to the geographic area and the type of food consumed [5–7]. Although great individual variations have been reported, the gastrointestinal tract represents

the main route of entry [8]. Strontium is mainly excreted by the kidney [6], suggesting that patients with renal insufficiency are at an increased risk for accumulation of the element. The distribution of Sr is similar to Ca, with 99% of the element being stored in bone [9]. Some published data suggest that in addition to effects on bone formation [10, 11], resorption [10–12], and mineralization [10, 11, 13, 14], there may be interference by Sr with Ca metabolism [6, 9] and vitamin D synthesis [15, 16]. The proposed effects of Sr on bone might be of particular interest in patients with chronic renal failure, especially those treated by dialysis. Indeed, Sr may accumulate in these patients because of the impaired renal function, the contamination of phosphate binders, and when treated by dialysis, the use of Sr containing parenteral and dialysis fluids [17, 18]. The poverty of information regarding the element's metabolism is mainly due to the lack of reliable methodologies able to accurately determine and localize the element at the relative low levels in which it is present in the human body.

To support the previous findings of the bone biopsy study, a worldwide multicenter study was established to check whether certain dialysis patients or centers are at an increased risk for Sr accumulation, and to study which factors are responsible for the accumulation of the element within this population. In this study using a recently developed electrothermal atomic absorption method [19], (*a*) serum Sr levels in subjects with normal renal function, preterminal renal failure, and hemodialysis (HD) were determined and compared with each other, (*b*) serum Sr levels of dialysis patients coming from different centers located in various countries were compared, (*c*) the efficacy of the water treatment procedure in the removal of Sr during the preparation of the dialysis fluids in the participating centers was checked, and (*d*) the possible role of various factors in the accumulation of the element was studied.

METHODS

Patients

Subjects with normal renal function. Serum Sr levels were determined in 24 subjects with normal renal function who had a median age of 33 years (range 22 to 40). Thirty-three percent were male subjects.

Patients with stable renal failure. In 75 patients with stable chronic renal failure and who were not yet on dialysis, we also determined serum Sr levels. Forty-eight percent of the patients in this group were male; the median age of these patients was 54 years (range 18 to 86). Based on the creatinine clearance, patients were divided into two groups (creatinine clearance < and >50 ml/min).

Patients treated by HD. Eight hundred and thirty-four HD patients coming from 17 European centers, 9 South and Central American centers, 1 Middle-Eastern center,

1 Japanese center, and 6 African centers were included in this multicenter study. Serum samples were taken before the start of the HD session. Besides the Sr level in serum, we also determined the element's concentration in tap water, water samples taken at several points of the water-purification process, and the final dialysis fluid. In addition to Sr, the Al concentration in all serum and water samples, as well as in the final dialysis fluid, was determined also.

A questionnaire with personal and clinical data from each dialysis patient was completed, together with information about the dialysis modalities of each center. The dialysis population had a median age of 56 years (range 5 to 96), a median time on dialysis of 36 months (range 1 to 300), and a 55% male predominance. A Sr-rich diet (seafood and grains) was ingested by 65% of the population. Concerning the phosphate binders: 56.6% of the patients took only $CaCO_3$; 4.9% of the subjects took only $Al(OH)_3$, and 13.0% of the total population took a combination of $Al(OH)_3$ and $CaCO_3$ and/or other phosphate binders. Some of the patients (25.5%) took no phosphate binder or none of the types of phosphate binders we asked about in our questionnaire. Vitamin D supplements were used by 57.2% of the patients. A majority of the patients (57.1%) received erythropoietin (EPO) treatment, and 54.5% underwent transfusions. Only 1.6% of the patients were treated by deferoxamine (DFO) therapy, whereas 2.6% had undergone a parathyroidectomy.

Strontium and Al in serum, water, and dialysis fluids were analyzed using a Zeeman 3030 atomic absorption spectrometer equipped with an HGA-600 graphite furnace, an AS-60 autosampler, and an Anadex DP-9500B silent scribe printer, all from Perkin-Elmer (Norwalk, CT, USA). To determine Sr, all samples were diluted fourfold in a solution of 0.5 ml/liter Triton X-100 and 1 ml/liter HNO_3. Aluminum in serum was determined after a 1:3 dilution of the samples in reversed osmosis (RO) water. Interassay coefficient of variance (CV) was <6% ($N = 12$, $N = 10$, respectively), and the recovery of added analyte was close to 100% for all of the biological matrices under study. Methods for the determination of Sr and Al have been described by us in detail previously [19, 20]. In order to reduce the risk for contamination, Sr- and Al-free material for sampling and sample storage was sent to each of the participating centers. All serum, water, and dialysate analyses were performed in a single laboratory, that is, the Department of Nephrology/Hypertension of the University of Antwerp.

Statistics

Data were analyzed using the statistical package SPSS for Windows. Data are expressed as mean ± SD. Univariate analysis was performed with *t*-test and chi-square test. The *a priori* level of significance was set at $P \leq 0.05$. For multivariate analysis, multiple linear regression and
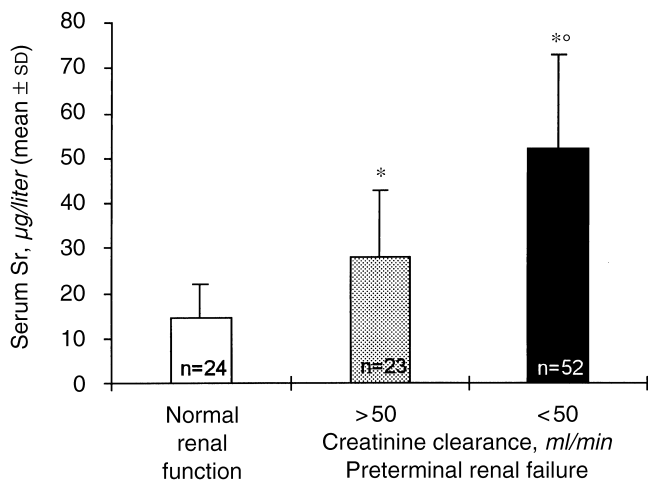
Fig. 1. Serum strontium (Sr) levels in subjects with various degrees of renal function. $*P < 0.001$ vs. normal renal function; $°P < 0.001$ vs. creatinine clearance >50.

logistic regression were used. The squared correlation coefficient (R^2) was calculated for evaluating the explained proportion of variability in a model. Odds ratios and 95% confidence intervals (95% CI) were estimated using the exp (B) of the logistic regression equation.

RESULTS

The mean $\pm$ SD serum Sr level in individuals with normal renal function was 14 ± 7.6 µg/liter. In the group of patients with a creatinine clearance of more than 50 ml/min, the mean serum Sr level was 28 ± 15 µg/liter. In the group of patients with preterminal renal failure (creatinine clearance <50 ml/min), the mean serum Sr level was 52 ± 21 µg/liter. The significant increase ($P < 0.0001$) of the serum Sr level with decreasing renal function is shown in Figure 1.

The overall mean $\pm$ SD serum Sr level of the HD patients under study was 95 ± 103 µg/liter. A center-to-center comparison of the mean serum Sr level of all dialysis patients indicated significant differences ranging from a mean $\pm$ SD value of 25 ± 8 µg/liter in the center with the lowest value up to 466 ± 90 µg/liter ($P < 0.0001$) in the center with the highest level (Fig. 2). According to the mean Sr level, the dialysis centers can be categorized into two groups: a first group of centers with Sr levels below 100 µg/liter (low Sr group, 42 ± 19 µg/liter) and a second group of centers with levels above 100 µg/liter (high Sr group, 231 ± 104 µg/liter). Of interest was that all dialysis centers in the latter group were located in developing countries. As shown in Figure 3, the distribution of the serum Sr levels in both groups indicates only a very small overlap (0.72%) between groups. These data document that nearly all patients from dialysis centers in the high Sr group will have increased serum Sr

levels. Figure 4 shows the mean serum Sr levels in the South and Central American dialysis centers that participated in the multicenter study. Although most dialysis centers in this region have increased Sr levels, we cannot generalize this because levels in both Chile and Argentina are comparable to the concentrations of the element found in Western countries.

Another interesting finding was that in the high-Sr group, increased Sr levels were accompanied by increased levels of Al, that is, 72 ± 63 µg/liter compared with 19 ± 22 µg/liter in the low-Sr group. Serum Ca was significantly decreased in the high-Sr group (4.35 ± 0.57 mEq/liter) in comparison to the low-Sr group (4.65 ± 0.67 mEq/liter; Fig. 5). Also in Figure 5, the differences in personal and clinical factors between both groups are shown. Multivariate analysis of all of these data indicates these factors can explain 20% of the differences in serum Sr levels between both groups.

Out of this relatively small percentage, it is obvious that other factors must play a role in the accumulation of Sr in dialysis patients. To further explain this issue, we assessed whether the observed differences between the high and low Sr groups could be due to center-to-center variations in either tap water or dialysate. Assessing the evolution of Sr levels during the various steps of the water purification process, data indicated Sr levels in the tap water to differ greatly from center to center. During the water purification process, however, Sr was removed adequately from the water in both groups (Fig. 6). In the low-Sr centers, this results in a low concentration of the element in the final dialysis fluid. In the high-Sr group, however, an increase in the Sr concentration between points 4 (after reversed osmosis) and 5 (inlet artificial kidney) of the water treatment process was noted, resulting in an increased concentration of the element in the final dialysis fluid. It can be assumed that this accumulation is due to the addition of concentrates contaminated with Sr. Indeed, analysis of the concentrates used in certain dialysis centers of the high-Sr group revealed these solutions to be contaminated with Sr. Interestingly, the contamination occurred only in acetate-based concentrates (up to 15750 µg/liter; undiluted concentrate), whereas Sr levels in bicarbonate-based concentrates were below the analytical detection limit (<3 µg/liter). A strong correlation ($r = 0.74$, $P < 0.001$) between mean serum and dialysate Sr levels was noted. When the Sr concentration in the final dialysis fluid was assessed in a multivariate model, it explained 50% of the difference in Sr levels between both groups. Interestingly, when considering only the high-Sr group, other factors also seem to play a substantial role in the accumulation of the element, specifically, the serum Ca concentration, the use of vitamin D supplements, and the consumption of seafood contributed to 43.1% of the variation in serum Sr levels between dialysis patients within the high-Sr
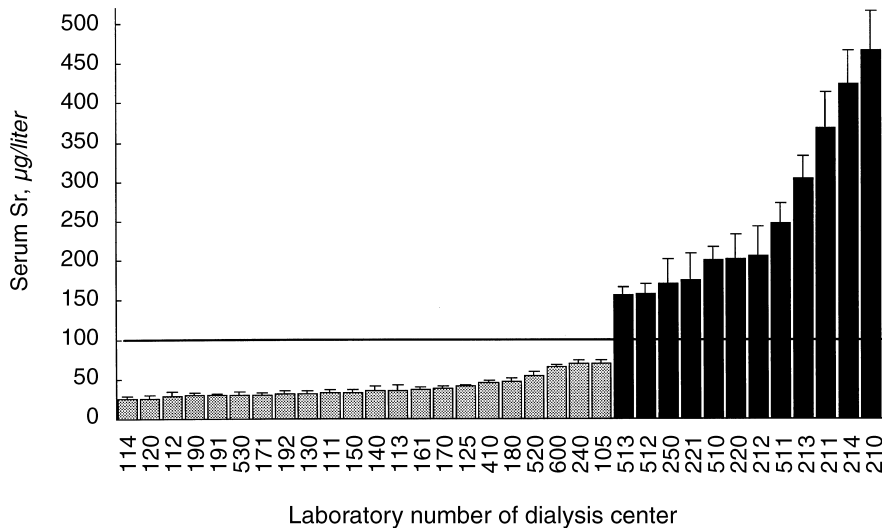
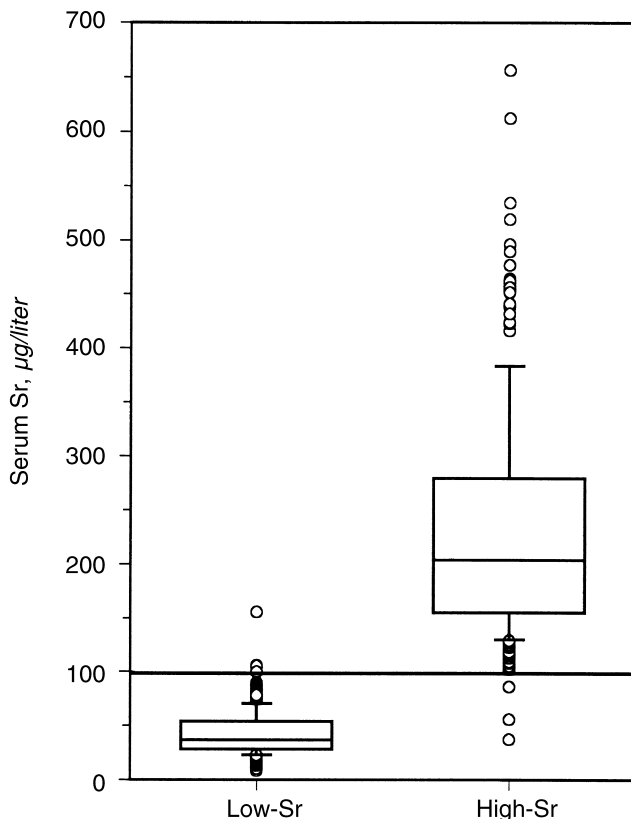**Fig. 2. Mean serum strontium (Sr) levels of dialysis patients from the different dialysis centers.**

Fig. 3. Distribution of serum strontium (Sr) levels in the low-Sr group versus the high-Sr group. There were 599 low-Sr patients [outliers ≥100 μg/liter: 0.15% (N = 3: 105, 106, 155 μg/liter)] and 235 high-Sr patients [outliers ≤100 μg/liter: 1.3% (N = 3: 37, 56, 86 μg/liter)]

group. No correlation was present between Sr and Al within the high-Sr group, indicating that in a number of these centers, high Sr levels were associated with low levels of Al.

DISCUSSION

As we previously reported, in a histological/chemical survey of 100 bone biopsies from dialysis patients originating from several centers in various countries, increased Sr concentrations as well as Sr/Ca ratios were observed in the bone of patients suffering from osteomalacia as compared with all other types of renal osteodystrophy [3]. Interestingly, a recent epidemiological study in Turkey demonstrated a significantly higher prevalence of the clinical diagnosis of rickets in children with normal renal function who were living in a region with a high soil Sr content and where nutrition was mainly based on cereals as compared with that in children living in a low soil Sr region [21]. Experimentally, it has been shown that administration of Sr to animals with normal renal function may result in a bone lesion with a histological resemblance to that seen in vitamin D-deficient rickets [22]. However, the so-called Sr-induced rickets [23, 24] differs from Ca- or phosphorus-deficiency rickets in that vitamin D supplements do not correct the lesion [24, 25]. In an experimental study in chronic renal failure rats loaded with Sr, we could also demonstrate the presence of histological lesions that were highly comparable with the histological features of osteomalacia seen in dialysis patients [4].

Our results clearly show that serum Sr levels are increased in subjects with decreasing renal function, as compared with individuals with normal renal function. This is not unexpected, as the element is mainly excreted by the kidney.

As early as 1973 using x-ray fluorescence, Rudolph, Alfrey, and Smythe noted distinctly (five- to eightfold) increased Sr concentrations in skeletal muscle of dialyzed uremics compared with those of nondialyzed individuals and controls that they suggested to originate from
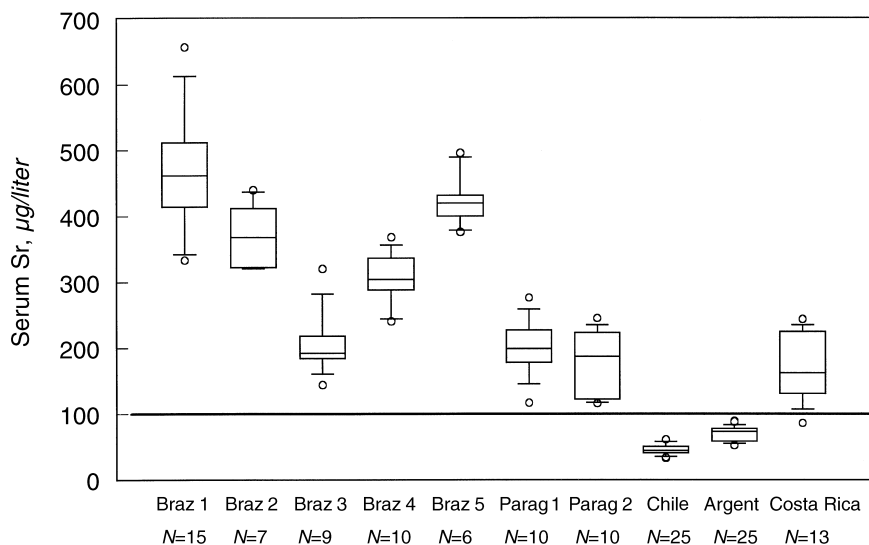
Fig. 4. Serum Sr levels in the South and Central American dialysis centers.

	Low-Sr centers ≤100 µg/liter N=599	High-Sr centers >100 µg/liter N=235
Serum Sr µg/liter	42.3 ± 18.7	231.0 ± 104.3
Serum Al µg/liter	18.9 ± 21.7	71.6 ± 63.0
Serum Ca mg/liter	4.65 ± 0.67	4.35 ± 0.57
Age years	57.4 ± 15.6	47.3 ± 15.3
Time in dialysis months	60.2 ± 53.5	34.7 ± 34.9
P-binder CaCO$_3$ %	51.6	68.7
Al(OH)$_3$ %	4.2	6.3
Comb. %	17.6	2.0
None %	26.6	23.0
Vit. D suppl. %	52.2	69.2
EPO %	63.8	40.8
Transfusions %	46.3	74.4
DFO %	1.0	4.0
PTX %	3.5	0.4
Seafood + grains %	74.0	42.9

Multivariate analysis: 20%
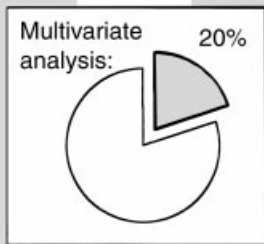
Fig. 5. Patient characteristics in the high-Sr and low-Sr groups.

the use of untreated high Sr tap water in the region of Denver that was used to prepare the dialysis fluid [26]. At that time, they suggested that in addition to Al, Sr might play a role in the development of renal osteodystrophy in dialysis patients. A contributive role for Sr and Al in the development of dialysis osteomalacia was also suggested by Canavese et al [17]. In this respect, epidemiological findings of our bone biopsy study, demonstrating a good correlation between Al and Sr in the osteomalacic patients but not in those presenting the other types of renal osteodystrophy, are of particular interest [3]. These findings could be confirmed in our recent epidemiological survey, where in the serum of dialysis patients from the

high-Sr group, besides the mean Sr levels, mean Al levels were also increased as compared with the low-Sr group.

Data of this multicenter study indicate that patients of particular dialysis centers are at an increased risk for Sr accumulation. We found Sr levels in dialysis patients to differ significantly from center to center, as well as from country to country. These great variations in Sr levels appeared to be mainly due to the use of Sr-contaminated dialysis fluids. High Sr levels in the dialysate seemed to originate from the addition of Sr-containing concentrates, particularly acetate-based concentrates, which are added to prepare the final dialysis fluids. Other factors, such as time on dialysis, vitamin D supplements,
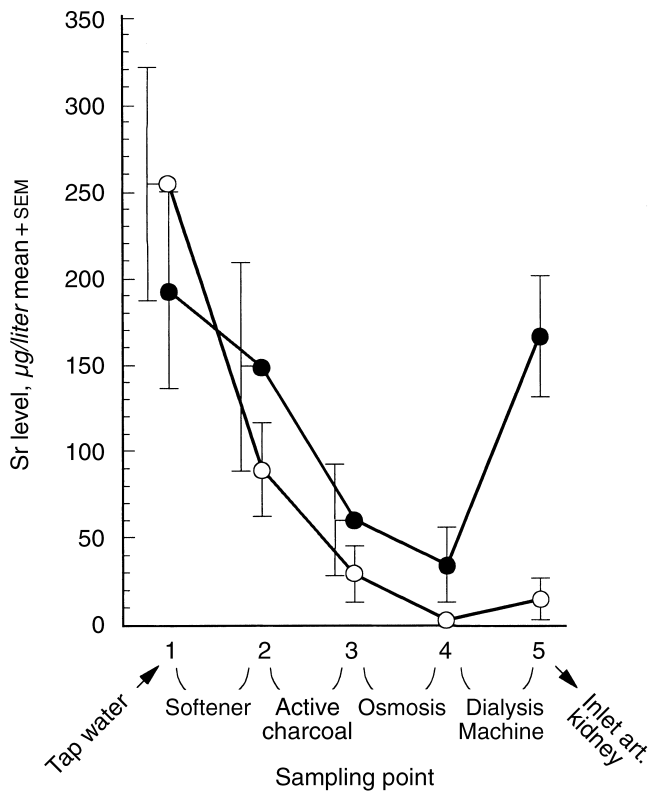
Fig. 6. Evolution of the strontium levels during the different steps of the water purification process in both the high (●) and low (○) Sr groups.

or type of phosphate binder used, seem to play a less important role in the accumulation of the element. With regard to the latter, Canavese et al suggested the oral intake of Al-containing phosphate binders, particularly Maalox® tablets, which may contain up to 217 µg/g of the element, to be an important source of Sr within the dialysis population [17].

Serum Ca levels were decreased in the high-Sr group as compared with the low-Sr group. This may point to an effect of Sr on Ca absorption and, as such, confirm previous observations of other groups, indicating that high Sr concentrations negatively affect Ca absorption, either directly or indirectly, possibly through the element's interference with vitamin D synthesis [16, 25].

Although the prevalence of osteomalacia has decreased in the overall dialysis population, the disease is still regularly observed in developing countries. In this context, it is of interest that we only found increased serum Sr concentrations in patients living in these countries. Hence, for these individuals, Sr accumulation may present an increased risk for the element's toxic effects. One of the centers in this multicenter study also participated in the previous bone biopsy study [3]. Out of the five patients in whom a bone biopsy was taken, four had the histological features of osteomalacia in the presence

of increased bone Sr levels. As noted in this study, all patients of that particular center also have increased serum Sr levels, which appeared to be due to the use of a high Sr dialysate (as high as 243 µg/liter). Hence, these data indicate that dialysis patients treated with Sr contaminated dialysis fluids are at an increased risk for Sr accumulation and perhaps the development of osteomalacia. Further studies are necessary, however, to clarify the relationship between Sr and osteomalacia.

Data of this multicenter study allow us to conclude that patients of particular dialysis centers are at an increased risk for Sr accumulation, which appears to be mainly due to Sr contamination of the dialysis fluid. To what extent this Sr accumulation has clinical consequences remains to be elucidated.

ACKNOWLEDGMENTS

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