Phosphate Salivary Secretion in Hemodialysis Patients: Implications for the Treatment of Hyperphosphatemia

Vincenzo Savica, Lorenzo A. Calò, Renato Caldarera, Adelaide Cavaleri, Antonio Granata, Domenico Santoro, Rodolfo Savica, Ugo Muraca, Agostino Mallamace, Guido Bellinghieri

Departments of nephrology and physiology, University of Messina, nephrology and dialysis units, Papardo Hospital, Messina, department of clinical and experimental medicine, Clinica Medica 4, University of Padua, Padua, Italy

Key Words
Hyperphosphatemia • Phosphate, salivary

Abstract
Background/Aims: Hyperphosphatemia is recognized as contributing to the increased risk of cardiac death in end-stage renal disease (ESRD) and hemodialysis (HD) patients. Currently available pharmacologic treatment for hyperphosphatemia is based on phosphate binders but, despite treatment, only half of the patients fall within the range for serum phosphorus of the K/DOQI guidelines. Therefore, there is a need to identify other therapeutic approaches in order to reduce serum phosphate. Salivary fluid contains phosphate which, if related to the daily salivary secretion (1,000–1,880 ml), may raise interest in order to identify further additive approaches to phosphorus removal in uremic patients, while data about salivary phosphate secretion in ESRD patients are controversial. Methods: This study evaluates salivary phosphate secretion in 68 HD patients compared with 30 healthy subjects. Saxon’s test confirmed normal salivary function in patients and controls. Salivary calcium and serum phosphate, calcium and PTH were also measured. Results: HD patients had significantly higher salivary phosphorus levels compared with healthy controls: 30.35 (26.5–34.6) vs. 12.1 (10.58–14.73) mg/dl (p < 0.0001), and this significantly correlated (p < 0.0001) with serum phosphorus. Multiple regression analysis confirmed serum phosphorus as the only predictor (p < 0.0001) of salivary phosphorus. Conclusions: Given the functional secretory similarity between salivary glands and the kidneys, this increased salivary phosphate secretion might be interpreted as being compensatory in the presence of renal failure. Absorption of the increased salivary phosphate secretion, however, may worsen hyperphosphatemia; therefore, the binding of salivary phosphate might be considered as a further therapeutic approach to hyperphosphatemia in ESRD.

Introduction
The mortality risk of cardiovascular disease in patients with end-stage renal disease (ESRD) and in those under renal replacement treatment with dialysis is known to be 30 times higher than in the general population [1]. Hyperphosphatemia is a recognized factor contributing mainly to the increased risk of cardiac death in these patients [2, 3]. In fact, in patients with renal disease, the well-known relationship between hyperphosphatemia, secondary hyperparathyroidism, bone turnover and extra-osseous calcifications has recently led to the recogni-
tion of the major role played by elevated serum phosphate levels in the induction of vascular calcification [4-6], cardiac interstitial fibrosis and arterial thickening [7], therefore highly increasing the risk of cardiac death [2, 3]. Passive mechanisms responsible for vascular calcifications are thought to be due to the elevated phosphate plasma levels and high calcium phosphate ion product resulting in supersaturated plasma [8, 9]. In ESRD patients and in those under renal replacement treatment with dialysis, much attention has therefore been focused on therapeutic interventions for their abnormalities in mineral and phosphate metabolism in order to reduce the impact of these important determinants of vascular calcifications on the increased risk of cardiac death [10].

With regard to hyperphosphatemia, other than a reduction in dietary phosphorus intake and dialysis treatment, the currently available pharmacologic therapy for hyperphosphatemia is based on phosphate binders, including the recently introduced lanthanum carbonate and sevelamer [11]. However, despite this treatment, only 45% of the patients fell within the range for serum phosphorus of the K/DOQI guidelines [12].

Treatment with phosphate binders is essentially used to limit dietary phosphorus absorption by binding dietary phosphorus during passage through the intestinal tract. No other way for phosphorus removal, except dialysis, has been found.

Normal salivary secretion is known to be in the range of 1,000-1,800 ml/day [13]. Salivary fluid contains electrolytes including phosphate [13] which, if related to the amount of salivary secretion per day, may raise interest in identifying another possible approach to phosphorus removal in uremic patients. There is, however, little information on the phosphorus content of salivary secretions and the salivary phosphate secretion in ESRD patients.

This study was set up to evaluate the salivary phosphate secretion in a group of ESRD patients under chronic dialysis treatment compared with healthy subjects used as control group. The results of this study and, in particular, the relationship between increased salivary phosphate and phosphatemia, shown for the first time by this study, might open the way for an additional therapeutic approach to the treatment of hyperphosphatemia in these patients through the removal of excess salivary phosphate.

Patients and Methods

Sixty-eight ESRD patients from the Dialysis Unit at the Papardo Hospital, Messina (47 males and 29 females, mean age 61.6 ± 9.4 years) on 240-min three times a week bicarbonate dialysis with polysulphone dialyzers (HD patients) were enrolled in the study. Thirty healthy subjects (18 males and 12 females, mean age 52.3 ± 6.4 years) from the medical and paramedical staff of our division were used as controls.

Inclusion criteria were age older than 18 years and a minimum of 1 year of hemodialysis treatment. Exclusion criteria were acute infections, malignancy, inflammatory processes, dry mouth symptoms and the presence of clinical signs of autonomic nervous system impairment according to our previously reported protocol [14] as well as patients with Sjögren's syndrome.

All patients and controls were checked for normal salivary function using the Saxon test [15] at enrollment, and all patients were treated with the phosphate binder, sevelamer, at a dose of 3.2-4.8 g/day.

All patients gave an informed consent before participation and the study was approved by our institutional authorities.

Serum phosphorus, calcium and parathyroid hormone (PTH) were measured from fasting predialysis blood samples in HD patients. In control subjects, blood samples were collected from an antecubital vein.

Before the hemodialysis session, 2 ml of saliva were collected by direct suction from the oral vestibule using an automatic pipette. After centrifugation of the sample, calcium and phosphorus concentrations were evaluated. The same determinations were performed in the control subjects.

After centrifugation of the sample, serum and salivary calcium and phosphorus levels were determined in a spectrophotometric assay using a flex reagent cartridge (Dade Behring Inc., Newark, N.J., USA). The serum PTH concentration was evaluated using the Immulite 2000 intact PTH, solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay (Diagnostic Product Corporation, Los Angeles, Calif., USA), with normal values ranging between 7.0 and 33.0 pg/ml.

Statistical Analysis

Data were evaluated on a Macintosh G5 computer (Apple Computer, USA) using the Statview II statistical package (Brain-Power Inc., USA).

The distribution of the data was established with the Kolmogorov-Smirnov test. A normal distribution resulted only for PTH. Given the skewed distribution of serum and salivary calcium and phosphorus in HD patients, a logarithmic transformation was performed before statistical analysis. Data are expressed as geometric means with 95% confidence intervals, and were analyzed using the non-parametric Wilcoxon-Mann-Whitney rank sum test. Values at the 5% level or less (p < 0.05) were considered statistically significant.

Regression analysis was used for HD patients to identify relevant relations between levels of salivary phosphate and other parameters while multiple regression analysis was performed in order to verify independent predictors.

Results

In HD patients, the serum and salivary calcium levels were 8.73 (8.49-8.98) and 7.21 (6.58-7.91) mg/dl, respectively. In these patients the serum and salivary phos-
Fig. 1. Relationship between serum and salivary phosphorus in HD patients.

Phosphorus levels were 5.56 (5.24–5.91) and 30.27 (26.50–34.58) mg/dl, respectively. PTH was 143.63 ± 99.41 pg/ml.

In control subjects the salivary phosphorus level was 12.1 (10.58–14.73) mg/dl.

Considering the upper limit of the confidence intervals for the mean salivary phosphate as the cutoff in control subjects (14.73 mg/dl), 62 of 68 subjects (91.17%) had an increased salivary phosphate level.

In HD patients, salivary phosphorus was significantly higher compared with healthy controls: 30.35 (26.5–34.6) vs. 12.1 (10.58–14.73) mg/dl (p < 0.0001).

The salivary phosphorus levels of HD patients correlated significantly (p < 0.0001) with the serum phosphorus levels (fig. 1).

Multiple regression analysis confirmed that only serum phosphorus was significantly and independently correlated (p < 0.0001) with salivary phosphorus (table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.01</td>
<td>1.17</td>
<td>-</td>
</tr>
<tr>
<td>Serum log P</td>
<td>1.12</td>
<td>0.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum log Ca</td>
<td>-0.06</td>
<td>0.15</td>
<td>&lt;0.70</td>
</tr>
<tr>
<td>Salivary log Ca</td>
<td>0.69</td>
<td>0.49</td>
<td>&lt;0.16</td>
</tr>
<tr>
<td>PTH</td>
<td>0.0008</td>
<td>0.0006</td>
<td>&lt;0.19</td>
</tr>
</tbody>
</table>

Discussion

Decreasing the excess morbidity and mortality from cardiovascular disease is a target of primary importance in the therapeutic strategies for ESRD and HD patients. Cardiovascular calcifications are known to contribute greatly to the increased morbidity and mortality of ESRD and HD patients [1, 2] and hyperphosphatemia is recognized as playing a causative role in the induction of these complications [2–7]. To date, pharmacologic therapeutic approaches to hyperphosphatemia in ESRD patients are based only on phosphate binders which, together with reduced dietary phosphate intake, join dialysis to remove excess phosphorus from the blood. Despite these therapeutic measures, however, more than half of the patients still have serum phosphate levels above the range recommended by the K/DOQI guidelines [9]. Therefore, it is very important to identify new methods of intervention and other possible therapeutic approaches to reduce phosphate levels in blood.

Salivary secretion ranges between 1,000 and 1,800 ml/day and is a considerable source of electrolytes including phosphate [13]. The results of our study show that in our HD patients the salivary glands produce a relevant amount of phosphate. In fact, we observed that, compared with healthy controls, the salivary phosphorus ratio in HD patients is more than doubled and salivary phosphorus is five times higher than serum phosphorus. In addition, in HD patients, salivary phosphorus correlated with serum phosphorus, and multivariate analysis confirmed that serum phosphorus was the only independent factor predicting increased salivary phosphorus secretion. In our patients we did not measure the total amount of saliva secreted; however, in consideration of the normal salivary function of the patients enrolled in our study given their normal values on the Saxon test, we can rationally assume that the salivary secretions of our patients range between the values for a normal salivary secretion reported above. Therefore, based on our data and according to the amount of daily saliva secretion, the salivary glands seem able to secrete a relevant amount of phosphate, which may range between 302.7 and 544.86 mg. In addition, this increased salivary phosphate secretion was found in almost all (91.73%) of our HD patients, further validating our results.
Recent studies have considered the function of salivary glands in dialysis patients with contrasting results [15–17]. Kao et al. [16] showed a significantly poorer salivary function in ESRD patients with oral manifestations compared with ESRD patients without oral manifestations and healthy controls. Postorino et al. [15] reported reduced salivary function in HD patients and attributed this to the presence of fibrosis of the salivary glands. In contrast, Blum et al. [17] reported increased salivary phosphate levels in ESRD patients, and related this to the secondary hyperparathyroidism of uremic patients. All the patients evaluated in our study were free of oral manifestations and had normal salivary function as confirmed by the Saxon test, therefore ruling out the presence of fibrosis of the salivary glands. In agreement with the data of Blum et al. [17] our patients had increased salivary phosphate; however, no relationship was found between the increased salivary phosphate secretion and PTH, which makes any influence of secondary hyperparathyroidism on salivary phosphate secretion unlikely. It must be noted that in 1979, at the time the report by Blum et al. [17] was published, measurements of PTH were based on the less sensitive evaluation of PTH fragments which, compared with the currently used evaluation of PTH through measurement of the whole molecule, could have overestimated the PTH concentration in those patients and were the basis for the evidence of a relationship between PTH and increased salivary phosphate.

The secretory units of salivary glands are clusters of cells called acini and their function is similar to that of renal tubules. The acini, in fact, secrete a primary salivary fluid that contains water, electrolytes, mucus and enzymes, which flow into the collecting ducts where the composition of saliva is managed. Sodium is actively reabsorbed while potassium and phosphate are secreted. In HD patients, the increased salivary secretion, salivary phosphate in particular, could be a rational explanation for the compensatory activity of the salivary glands increasing their secretion in the presence of impaired kidney function. The effect of this compensatory secretion is, however, abolished by the ingestion of saliva and its following absorption in the intestinal tract that may become a vicious circle between salivary phosphate secretion and fasting phosphate absorption, thereby worsening hyperphosphatemia.

The demonstration that salivary phosphate secretion in our HD patients is increased could open an important path for a possible additional therapeutic approach to hyperphosphatemia by breaking the vicious circle caused by the absorption of the increased salivary phosphate. In fact, by knowing the salivary phosphate balance in HD patients, it could be possible to bind salivary phosphate secretion, reducing its absorption, and therefore further reducing the serum phosphate concentration. This is particularly interesting when considering that all our study patients were treated with sevelamer as a phosphate binder and, notwithstanding, they still had hyperphosphatemia, there by confirming the need for further interventions.

References