Microscopic nephrocalcinosis in chronic kidney disease patients

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ABSTRACT

Background. Experimental data indicate that microscopic calcium phosphate deposition in the kidney (nephrocalcinosis) may accelerate progression of chronic kidney disease (CKD). Data on the prevalence, risk factors and implications of nephrocalcinosis in CKD patients are scarce. A mineral metabolism disorder could play an important pathogenetic role, as suggested by recent protocol biopsy findings in incident renal transplant recipients.

Methods. Kidney biopsy cylinders of CKD patients, collected between January 1989 and December 2007, were screened for the presence of nephrocalcinosis. Only patients with ≥ 1 parathyroid hormone (PTH) level available within 180 days of the biopsy were eligible for inclusion (n = 211). Demographics and mineral metabolism parameters were retrieved from medical files. Data on renal death (up to December 2012) were obtained from the Flemish ESRD registry. Baseline biopsies from 110 deceased kidney transplant donors served as controls.

Results. The prevalence of nephrocalcinosis in kidney donors and patients with CKD 1-2, CKD 3–4 and CKD 5-5D was 4.6, 14.3, 20.2 and 54.0%, respectively (P < 0.0001). Among CKD patients, patients with nephrocalcinosis were characterized by lower estimated GFR, lower serum bicarbonate level and higher serum PTH and calcium level. In multivariate regression analysis, high serum PTH, calcium and creatinine level, and low serum bicarbonate level were all significantly and independently associated with nephrocalcinosis. Serum phosphorus level, but not nephrocalcinosis predicted renal death, independent of renal function.

Conclusions. Our data demonstrate that prevalence rates of nephrocalcinosis increase with increasing CKD stage to reach more than 50% in end-stage renal disease patients and suggest that acid–base and mineral metabolism disturbances are implicated in its pathogenesis.

Keywords: CKD-MBD, nephrocalcinosis

INTRODUCTION

Mineral metabolism disorders are prevalent in chronic kidney disease (CKD), even in the early stages [1]. Mineral metabolism disorders detrimentally affect bone health and contribute to the high cardiovascular burden in CKD patients [2]. Several studies also report an association between mineral metabolism disorders and an accelerated loss of renal function [3–8]. According to the precipitation–calcification hypothesis, this accelerated loss may be explained (at least partly) by deposition of calcium phosphate (CaPhos) crystals in the renal parenchym, i.e. nephrocalcinosis [9, 10]. Besides the urinary calcium and phosphate load, the intratubular pH and the concentration of inhibitors such as citrate, magnesium, pyrophosphate and urinary proteins (e.g. fetuin-A) will determine whether precipitation of microcrystals will occur or not [9, 11–13].

Nephrocalcinosis leads to an inflammatory response and renal damage, inciting a vicious circle [12]. Dietary phosphorus restriction or phosphate-binders reduce the occurrence of nephrocalcinosis and attenuate the progressive loss of renal function, at least in animal studies [3, 9, 14–21].

Data on the prevalence, aetiopathogenesis and implications of nephrocalcinosis in humans are scarce. The renal calcium content of the kidney was reported to increase along the degree of functional renal impairment [19], to reach levels in CKD stage 5D that are 8-fold greater than that found in normal kidneys [22]. Nephrocalcinosis in renal transplant recipients is highly prevalent and related to disturbances of mineral metabolism [23, 24]. Preliminary evidence supports the hypothesis that nephrocalcinosis accelerates renal function deterioration in these patients [25, 26]. The present study aimed (i) to define the prevalence of renal calcium phosphate
deposition in CKD across stages of disease and (ii) to identify risk factors.

MATERIALS AND METHODS

Study design and population

We performed a single-centre retrospective observational study. All patients with stable renal function who underwent a biopsy or a nephrectomy of a native kidney at the University Hospitals Leuven between 1 January 1998 and 31 December 2007 were eligible for inclusion. Stable renal function was defined arbitrarily. In CKD patients not yet on dialysis, all serum creatinine values recorded within a predefined time frame, i.e. 15 days prior to 60 days after the biopsy, were assessed. Renal function was considered stable if the ratio of the highest to the lowest serum creatinine value within this time frame was <2. Dialysis patients were eligible for inclusion if maintenance dialysis was initiated at least 1 month before the biopsy/nephrectomy. Patients with no parathyroid hormone (PTH) levels available within the predefined time window (see below) were excluded from the analysis. Biopsy specimens from deceased kidney donors served as control [24]. Patients were followed prospectively until end-stage renal disease (primary end point), death, end of study (31 December 2012) or whatever came first.

Data collection

Relevant clinical data and biochemistry were retrieved from an automated database. Biochemical data obtained at the time of the biopsy or the nephrectomy or, if unavailable, at the most proxy time point within a predefined time window were registered. The predefined time window amounted to 180 days before and after the biopsy/nephrectomy for PTH and 14 days for creatinine, calcium (Ca), phosphate (Phos), alkaline phosphatases and albumin. In dialysis patients, only predialysis determinations were recorded. Serum concentrations of PTH were determined by a uniform immunoradiometric assay (IRMA), as described elsewhere [27]. In contrast to most other commercially available IRMAs for PTH, this assay detects full-length human PTH but not N-terminal truncated fragments, and thereby resembles recently introduced third-generation PTH IRMAs (biointact PTH or whole PTH). This also explains its lower normal range of 3–40 pg/mL [ng/L]. The estimated glomerular filtration rate (eGFR) was calculated using the short Modification of Diet in Renal Disease formula [28]. Data on renal death (up to December 2012) were obtained from the Flemish ESRD registry (NBVN).

Histopathological analysis

Two tissue cylinders were screened for calcifications as previously described [24]. Briefly, one cylinder was snap frozen in liquid nitrogen-cooled 2-methyl-butane and the second cylinder was fixed in buffered formalin. Slides containing 4–10 paraffin sections (2–3 µ), were routinely stained with haematoxylin–eosin (HE), with periodic acid schiff (PAS) and with a silver methenamine method [29]. Only the HE-stained slides of both fixed and frozen tissue were used to look for calcifications, as in the PAS and silver methenamine stains, the first processing step is incubation of the slide in periodic acid which may dissolve calcifications. The renal histopathological analysis of the calcifications was performed in a blinded fashion.

Statistics

Parametric and non-parametric parameters are expressed as mean ± SD and median [interquartile range], respectively. Differences between groups were analysed using ANOVA. Logistic regression analyses were used to examine the associations between parameters. The SAS version 9.3 (SAS Institute, Cary, NC, USA) software programme was used for the statistical analysis. Two-sided P < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Patient disposal is depicted in Figure 1. Two-hundred and eleven CKD patients (M/F 124/77, mean age 54.5 ± 13.5 years) were enrolled in the present study. Relevant clinical and biochemical data are summarized in Table 1. Primary renal disease was diabetes in 7.6%, vasculitis/glomerulonephritis in 46.0%, interstitial diseases in 5.7%, congenital/hereditary/cystic in 8.5%, vascular disease in 1.0%, miscellaneous in 13.3% and unknown in 18.0%. Fifteen samples originated from nephrectomy specimens (ADPKD n = 9, tumour n = 5, chronic pyelonephritis n = 1). Acid–base and mineral disturbances across CKD stages were as expected [30]. Deceased kidney donors (n = 110, M/F 53/57, mean age 45.2 ± 15.2) served as controls.

Prevalence of nephrocalcinosis

Nephrocalcinosis (Figure 2) was observed in 4.6, 14.3, 20.2 and 54.0% of kidney tissue samples obtained from controls, CKD1–2, CKD3–4 and CKD5–5D patients (P < 0.0001) (Figure 3). Calcium oxalate (CaOx) deposition was excluded in all but one sample by means of a Pizzolato stain. The prevalence of nephrocalcinosis in CKD patients not yet on dialysis
Table 1. Demographics and biochemistry across CKD stages

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>CKD</th>
<th>CKD1-2</th>
<th>CKD3-4</th>
<th>CKD5-5D</th>
<th>P (CKD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>110</td>
<td>211</td>
<td>42</td>
<td>119</td>
<td>50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>45.2 ± 15.2</td>
<td>56.8 ± 16.0</td>
<td>43.8 ± 15.1</td>
<td>60.5 ± 14.2</td>
<td>59.0 ± 15.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Gender, Male (%)</strong></td>
<td>48.0</td>
<td>69.1</td>
<td>81.0</td>
<td>66.4</td>
<td>66.0</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>PO4 (mg/dL)</strong></td>
<td>NA</td>
<td>4.2 ± 1.5</td>
<td>3.6 ± 0.6</td>
<td>3.8 ± 0.9</td>
<td>5.6 ± 2.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Albumin (g/dL)</strong></td>
<td>NA</td>
<td>33.7 ± 8.2</td>
<td>32.2 ± 10.0</td>
<td>34.3 ± 7.9</td>
<td>33.5 ± 7.2</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Ca (mg/dL)</strong></td>
<td>NA</td>
<td>8.9 ± 0.8</td>
<td>8.8 ± 0.7</td>
<td>8.9 ± 0.8</td>
<td>9.1 ± 0.9</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Bicarbonate (mEq/L)</strong></td>
<td>NA</td>
<td>24.4 ± 3.7</td>
<td>26.8 ± 2.5</td>
<td>23.8 ± 3.7</td>
<td>23.7 ± 3.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Metabolic acidosis (%)</strong></td>
<td>NA</td>
<td>26</td>
<td>2</td>
<td>30</td>
<td>34</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Bi-PTH (ng/L)</strong></td>
<td>NA</td>
<td>29.1 [10.0–60.7]</td>
<td>10.6 [3.6–18.6]</td>
<td>29.3 [10.0–45.7]</td>
<td>69.6 [43.3–141.5]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Total alkaline phosphatases (IU/L)</strong></td>
<td>NA</td>
<td>222 ± 118</td>
<td>216 ± 142</td>
<td>224 ± 115</td>
<td>226 ± 105</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Creatinine (mg/dL)</strong></td>
<td>0.8 ± 0.2</td>
<td>3.1 ± 2.4</td>
<td>1.1 ± 0.2</td>
<td>2.3 ± 0.8</td>
<td>6.5 ± 2.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>eGFR mL/min/1.73 m²</strong></td>
<td>NA</td>
<td>36.0 ± 24.7</td>
<td>75.9 ± 14.0</td>
<td>32.7 ± 12.2</td>
<td>10.2 ± 5.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The prevalence rate did not differ between patients with (8.8%) and without (5.8%) nephrocalcinosis.

**Determinants of nephrocalcinosis**

Clinical and mineral metabolism parameters were compared between CKD patients with and without CaPhos deposition (Table 2). Patients with nephrocalcinosis were characterized by lower kidney function, lower serum bicarbonate level and higher serum PTH and calcium level. Table 3 demonstrates factors associated with nephrocalcinosis in CKD patients. In univariate analysis, high serum phosphorus, high PTH, high creatinine and low bicarbonate were all significantly associated with nephrocalcinosis. In multivariate analysis, high serum calcium (not phosphorus), high PTH, high creatinine and low bicarbonate were found to be independently associated with nephrocalcinosis. After exclusion of dialysis patients, the same variables but serum creatinine remained in the final multivariate model.

**Mineral metabolism, nephrocalcinosis and renal outcome**

Mean follow-up in predialysis patients was 74.5 ± 48.7 months. The primary end point of ESRD was reached by 71 (40.3%) patients. In multivariate regression analysis including serum creatinine, bicarbonate, parameters of mineral metabolism and nephrocalcinosis, serum creatinine (OR 2.25, 95%CI 2.25–3.29, P < 0.0001) and serum phosphate (OR 1.60, 95%CI
The findings of the present study demonstrate that nephrocalcinosis is prevalent in CKD patients and suggest that acid–base and mineral metabolism disturbances are implicated in its pathogenesis.

We observed microscopic nephrocalcinosis in 27.0% of CKD patients. None of the patients had a history of hyperoxaluria. Specific staining indicated that calcium deposits in these patients almost exclusively consisted of CaPhos crystals. The prevalence of nephrocalcinosis in CKD patients was several-fold higher than in non-uremic controls (4.6%) and increased along the progression of renal disease to exceed 70% in dialysis patients. Clinical studies exploring the prevalence and pathogenesis of nephrocalcinosis in CKD patients are scarce. Gimenez et al. [19] studied the relation between renal calcium content and renal impairment in 246 human renal biopsies. Five-fold greater calcium content was measured in biopsied patients with preserved renal function than in normal postmortem renal tissue. Also in the present cohort, the prevalence of nephrocalcinosis was already 3-fold higher in patients with CKD stage 1–2 (14.6%) when compared with non-uremic controls (kidney graft donors). Nephrocalcinosis thus not merely is a dystrophic phenomenon of advanced kidney disease.

The pathogenesis of nephrocalcinosis in CKD is incompletely understood. Mounting evidence points to a disordered mineral metabolism as a major culprit [19, 23, 24]. In the present study, both serum calcium and PTH were independently and directly associated with nephrocalcinosis. Hyperparathyroidism and hypercalcemia may cause the luminal calcium phosphate product to (periodically) exceed the limit of solubility. We furthermore identified an independent association between metabolic acidosis and nephrocalcinosis. Metabolic acidosis reduces renal citrate production and increases citrate reabsorption, thus reducing the luminal concentration of citrate, i.e. a major inhibitor of calcium phosphate precipitation [9]. The observation that adjustment for mineral metabolism parameters and bicarbonate attenuated, but did not eliminate the association between renal dysfunction and nephrocalcinosis suggests that other factors may also be involved. Additional candidate risk factors include low magnesium [31] and low fetuin-A [13] levels.

Importantly, in animal experiments phosphate load excreted per nephron rather than serum phosphate concentrations related to kidney calcification [9]. This strongly suggests that nephrocalcinosis results from intrarenal causes and that, as such, its pathogenesis does not mimic metastatic calcification in the vasculature and other organs. Based on current knowledge, the following sequence of events is suggested to occur in the kidney: (i) intratubular CaPhos precipitation, most probably within the thin limb of the loop of Henle; (ii) retention/trapping of CaPhos microcrystals by a preconditioned [32] distal tubular epithelium; (iii) internalization of
CaPhos microcrystals via endocytosis; and finally (iv) ‘translocation’ to the renal interstitium [12, 33].

Both metabolic acidosis [34, 35] and mineral metabolism disturbances [4, 6, 36] have been associated with progression of kidney disease. The underlying pathophysiological mechanisms are complex and only partially understood. Since CaPhos microcrystals may initiate an inflammatory and fibrotic response (similar to CaOx), it may be speculated that nephrocalcinosis is in the causal pathway between metabolic acidosis and mineral metabolism disturbances (including high FGF23 levels) and accelerated progression of CKD [9, 20, 23, 37, 38]. In agreement with previous studies, we observed an independent association between serum phosphorus level and the incidence of renal failure [5, 6, 39, 40]. In patients with primary hyperoxaluria, nephrocalcinosis on imaging has recently been shown to associate with increased risk of kidney failure [41]. Opposite to these investigators, we failed to find an association between microscopic nephrocalcinosis and renal failure. Presumably, larger studies are required to determine whether nephrocalcinosis in CKD patients confers an independent risk for renal failure.

Our study has several limitations, which mainly are related to the retrospective design of the study. These include missing information on other relevant risk factors of nephrocalcinosis and renal disease progression. More specifically, measurements of serum FGF23 and fetuin A and of urinary mineral metabolism parameters were not available. The assessment of these parameters is strongly encouraged in future studies addressing nephrocalcinosis. All biopsies/kidney tissue samples in the present cohort were taken for clinical reasons. This inherently implies selection bias, which for example is reflected by the overrepresentation of patients with glomerulonephritis as primary renal disease. Strengths of the study include the availability of parameters of mineral metabolism including biointact PTH levels in all patients and a mean follow-up exceeding 6 years. It is well established that PTH shows an important biological variation [42]. That we nonetheless observed an association with nephrocalcinosis, emphasises its robustness.

In conclusion, our data demonstrate that microscopic nephrocalcinosis is prevalent in CKD patients and suggest that acid–base and mineral metabolism disturbances are implicated in its pathogenesis. Additional studies are required to establish whether nephrocalcinosis confers and independent risk for renal failure in humans.

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CONFLICT OF INTEREST STATEMENT

A conflict of interest is declared by none of the authors. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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