Crystalline nephropathy due to 2,8-dihydroxyadeninuria: an under-recognized cause of irreversible renal failure

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Abstract

Background. 2,8-dihydroxyadeninuria (DHA) disease (also called 2,8 dihydroxyadeninuria) is a rare autosomal recessive disorder caused by complete adenine phosphoribosyltransferase deficiency and typically manifests as recurrent nephrolithiasis. Only rare cases of DHA nephrolithiasis have been reported from the USA. Herein, we report three American patients who developed DHA crystalline nephropathy leading to end-stage renal disease (ESRD) with recurrence in the allograft.

Methods. Three cases of DHA crystalline nephropathy were identified from the Renal Pathology Laboratory of Mayo Clinic. Detailed clinical and pathologic descriptions are provided.

Results. All three patients were Caucasian adults with no history of obstructive nephropathy. Two patients had no history of nephrolithiasis and one had a single episode of stones 36 years prior to presentation. All patients presented with severe renal failure with a mean serum creatinine of 7.5 mg/dl. Renal biopsies revealed numerous tubular and interstitial brown DHA crystals, tubular degenerative changes and moderate to marked tubulointerstitial scarring. All three patients progressed to ESRD, within 1 month following renal biopsy in two and after 9 months in one. All three patients underwent renal transplantation with early disease recurrence in three allografts in two patients.

Conclusions. DHA disease is an under-recognized condition that can lead to irreversible renal failure and frequently recurs in the transplant. It should be included in the differential diagnosis of crystalline nephropathy, even in the absence of history of nephrolithiasis.

Keywords: 2,8-dihydroxyadeninuria; adenine phosphoribosyltransferase; crystalline nephropathy; disease recurrence; renal failure

Introduction

Adenine phosphoribosyltransferase (APRT) deficiency is a rare autosomal recessive inherited disorder of purine metabolism. APRT catalyzes the formation of adenosine monophosphate from adenine. In the absence of APRT activity, adenine is catabolized by xanthine oxidase to 2,8-dihydroxyadenine (DHA), which is excreted in the urine. DHA is insoluble in the urine at the physiological range of pH which may lead to crystalluria. Because there are individual differences in the ability to supersaturate the urine with DHA, the clinical manifestations vary among homozygotes from asymptomatic state to reddish-brown diaper stains in infants and to recurrent nephrolithiasis in children and adults [1]. Some patients develop chronic renal failure secondary to stone disease and recurrent urinary tract infection [1]. Very rarely, DHA crystals may deposit in tubular lumina and interstitium and lead to irreversible renal damage in the absence of history of nephrolithiasis [1–3]. Most cases of DHA disease reported in the literature were from Japan [4], Iceland [1] and France [5], with only five cases originating from the USA [6–10].

Unfortunately, awareness of DHA disease among urologists, nephrologists and pathologists in the USA is low [10]. DHA stones are radiolucent and are probably often misdiagnosed as uric acid stones since the standard chemical tests done on calculi do not distinguish between these two types. On the other hand, DHA crystals in renal biopsies may be confused with oxalate crystals as both are strongly birefringent and both deposit in tubular lumina, tubular cell cytoplasm and interstitium, resulting in an erroneous diagnosis of oxalosis [11]. Accurate diagnosis of DHA disease is crucial since early treatment with allopurinol effectively prevents further stone formation and may improve renal function in patients with parenchymal crystal deposition [1,2,10,12].

In this report, we describe three American patients with DHA disease who presented with renal insufficiency and were found to have chronic tubulointerstitial nephropathy with extensive crystal deposition. All three patients progressed to end-stage renal disease (ESRD), and two had early disease recurrence in the allograft documented on post-transplant biopsies. We provide detailed renal biopsy findings in these patients, which are lacking in the literature. This report will hopefully increase the awareness by...
both pathologists and nephrologists of this rare but under-
recognized cause of irreversible renal failure.

Materials and methods

Clinical histories (Table 1)

Case 1. A 38-year-old white woman with history of type 2 diabetes and depression presented in February of 2001 with weight loss, fatigue, nausea and vomiting. Workup revealed severe renal failure and anemia. She had no history of nephrolithiasis, obstructive nephropathy or pyelonephritis. Serum creatinine at presentation was 11.5 mg/dl, and serum albumin was 3.6 g/dl. She had no hematuria or proteinuria, and the urine sediment was inactive except for rare granular casts. CT scan showed no evidence of nephrocalcinosis or stones. A native biopsy was performed and showed chronic tubulointerstitial nephritis with extensive intratubular deposits of highly birefringent crystals thought to be oxalate crystals. She began hemodialysis 15 days after the biopsy. Her plasma oxalate levels were elevated, and she was started on pyridoxine for a suspected diagnosis of primary hyperoxaluria. An ophthalmology examination, however, did not show retinal oxalate deposition.

In January of 2002, she received a deceased donor renal transplant at an outside institution with immediate graft function, but at 3 days, she became anuric and did not recover allograft function. Two transplant biopsies performed 3 and 4 weeks post-transplant showed extensive tubular luminal and intracytoplasmic poliarizable crystals similar to those seen in the native biopsy. There was no evidence of rejection or calcineurin inhibitor toxicity. Hemodialysis was resumed in February of 2002. Plasma levels of oxalate were elevated but not to the degree expected in primary hyperoxaluria. A liver biopsy performed in October of 2003 revealed normal alanine glyoxylate aminotransferase activity and glyoxylate reductase activity, which excluded type 1 and type 2 primary hyperoxaluria.

In April of 2005, she received a second deceased donor renal transplant. Serum creatinine decreased to a nadir of 1.6 mg/dl 3 days post-transplant then slowly increased to 5.5 mg/dl at 6 months post-transplant. Recurrent disease was documented in allograft biopsies performed 3 days, 4 months, 5 months, 8 months and 1 year post-transplant. None of the biopsies showed acute rejection or calcineurin inhibitor toxicity. Urine oxalate levels were normal, though interpretation was complicated by her markedly reduced renal function. X-ray microanalysis of the tubular crystals, performed on the 4-month post-transplant biopsy, revealed that there was no calcium present and that the compound was organic, which would be consistent with a purine compound such as DHA. On re-review, the crystals seen in the native biopsy, the first allograft biopsies and the second allograft biopsies, were identical and typical of DHA crystals. The diagnosis of DHA disease was confirmed in November of 2005 by demonstrating complete absence of APRT enzyme activity on blood spots. The patient was started on allopurinol at a dose of 100 mg twice a day, at which time the serum creatinine was 4.0 mg/dl, along with a low-purine diet. The allograft function subsequently partially improved with decreasing creatinine to 2.6 mg/dl 10 months post-transplant. The 1-year post-transplant biopsy showed less tubular atrophy, interstitial fibrosis and inflammation than the 8-month biopsy, although both biopsies showed extensive DHA crystal deposition. In May of 2006, the patient developed hypotension and shock in the setting of toxic megacolon followed by acute deterioration of allograft function. During her difficult hospital course, allopurinol was temporarily discontinued. Renal allograft biopsy again showed extensive crystal deposition. Despite subsequent resumption of allopurinol, she remained dialysis dependent thereafter.

Case 2. A 35-year-old white male (the younger sibling of patient 1) was noted to have an elevated serum creatinine (3.9 mg/dl) in September of 1999 during an evaluation for complaints of chest pain. His baseline serum creatinine was known to be 1.1 mg/dl 6 months prior. He had a history of hypertension and diet-controlled type 2 diabetes mellitus but no history of nephrolithiasis and no stones were seen on CT scan. Urinalysis showed 1+ protein, mild leukocyturia and no casts, crystalluria or hema-
DHA crystalline nephropathy

Standard processing of the native renal biopsies included light microscopy (LM), immunofluorescence (IF) and electron microscopy (EM). For LM, all cases were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Mason's trichrome (TRI) and Jones methenamine silver (JMS). For IF, 3-μm cryostat sections were stained with polyclonal FITC-conjugated antibodies to IgG, IgM, IgA, C3, C1q, kappa, lambda, fibrinogen and albumin. Transplant biopsies were processed for LM and IF C4d staining and some were processed for EM.

In all three patients, activities of APRT and hypoxanthine phosphoribosyltransferase enzymes were assayed on blood spots with radiolabeled (C14) hypoxanthine and adenine using thin layer chromatography and liquid scintillation counting (University of California at San Diego Biochemical Genetics Laboratory).

The technique of energy-dispersive X-ray microanalysis was used to analyze the tubular crystals observed in the allograft biopsy in patient 1. Briefly, the tissue was first prepared for transmission EM by omission of osmium tetroxide and uranyl acetate, dehydration in graded alcohols and embedding in Spurr resin. Energy-dispersive X-ray microanalysis was done on unstained sections 70–90 nm in thickness, mounted on titanium grids, using an energy-dispersive X-ray spectrometer interfaced with an FEI Tecnai 12 transmission electron microscope. Spectra of the emitted energy of any element found were collected at an accelerating voltage of 80 kV and a specimen tilt of 25°.

Results

Native renal biopsy findings

The native renal biopsy findings in these three patients with DHA crystalline nephropathy are summarized in Table 2. Sampling for LM included a mean of 11.6 glomeruli, and a mean of 12% of glomeruli were globally sclerotic. In all cases, glomeruli were unremarkable without features of diabetic glomerulosclerosis. The most prominent histologic feature was acute and chronic tubulointerstitial nephropathy with acute tubular injury involving non-atrophic tubules, moderate to severe tubular atrophy and interstitial fibrosis and numerous DHA crystals. The crystals were diffusely present in tubular lumina and tubular cell cytoplasm as well as focally within the interstitium. They were seen predominately in the cortex and involved both proximal and distal tubules, with fewer deposits in the medulla. DHA crystals stained brownish-green to brown with the H&E and PAS stains, light blue on TRI and black on JMS (Figure 1A and B). They were strongly birefringent when examined under polarized light (Figure 1C). In the tubular lumina, they

<table>
<thead>
<tr>
<th>Table 2. Native renal biopsy findings</th>
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<tr>
<td>Patient 1</td>
</tr>
<tr>
<td>No. of glomeruli</td>
</tr>
<tr>
<td>Number of sclerotic glomeruli</td>
</tr>
<tr>
<td>% tubular atrophy and interstitial fibrosis</td>
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<tr>
<td>Interstitial inflammation</td>
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<tr>
<td>Vascular disease</td>
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<tr>
<td>Distribution of 2,8-dihydroxyadenine crystals</td>
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<td></td>
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<tr>
<td>Associated giant cells</td>
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</table>

Case 3. A 54-year-old white male was found to have renal failure and anemia in April of 2006 during a routine physical exam. His past medical history was significant for type 2 diabetes for 8 years, hypertension and obesity. He had no history of obstructive nephropathy or pyelonephritis. At the age of 18, he reported that he had passed six kidney stones but which were not analyzed at that time. He denied any subsequent episodes of nephrolithiasis. None of his close family members (father, mother, two brothers and two children) had history of renal failure or nephrolithiasis. On admission, serum creatinine was 7.0 mg/dl, and hemoglobin was 10 g/dl. Urinalysis revealed no proteinuria or hematuria. Renal ultrasound was negative for hydronephrosis, obstruction and nephrolithiasis. A renal biopsy was performed and showed chronic tubulointerstitial nephropathy with numerous tubulointerstitial crystals typical of DHA crystals. The diagnosis of DHA disease was confirmed by finding complete absence of APRT enzyme activity in the blood, and he was started on allopurinol 100 mg three times a day.

The patient began chronic dialysis in May of 2006 and received a deceased donor renal transplant in May 2007. He had delayed graft function but had a serum creatinine of 2.5 mg/dl at 1 month post-transplant. A renal biopsy performed 3 months post-transplant showed mild acute tubular necrosis and rare DHA crystals without rejection. Five weeks post-transplant, he developed abrupt worsening of serum creatinine to 4.5 mg/dl which was attributed to transplant ureteral stenosis accompanied by internal urine leak. A renal biopsy performed at that time also showed acute tubular necrosis but with abundant DHA crystals. After dilatation of stenosis and ureteral stent placement, serum creatinine declined to 2.5 mg/dl. In light of finding recurrent DHA disease, the allopurinol dose was increased to 300 mg twice a day. A repeat biopsy at 3 months post-transplant showed fewer crystals with minimal tubular atrophy, interstitial fibrosis and inflammation. At 18 months post-transplant, the patient had stable mild chronic renal insufficiency with a serum creatinine level that had further improved to 1.6 mg/dl.
Fig. 1. Pathologic findings in DHA crystalline nephropathy. (A) A low power view shows diffuse tubular degenerative changes with numerous intraluminal tubular DHA crystals. Focal interstitial inflammation, moderate interstitial fibrosis and two normal-appearing glomeruli are also seen (H&E, ×40). (B) At high magnification, intraluminal globular aggregates and rod-shaped intracytoplasmic brownish-green DHA crystals are evident (H&E, ×400). (C) The same field as Figure 1B is shown under polarized light. The DHA crystals are strongly birefringent (H&E, ×400). (D) In patient 3, focal large interstitial aggregates of annular formations of DHA crystals were identified (H&E, ×200). (E) On electron microscopy, the intraluminal DHA deposits form radially-oriented clusters of needle-shaped, electron-lucent crystals (×4200).
AGT, alanine/glyoxalate aminotransferase; GRHPR, glyoxylate reductase/hydroxypyruvate reductase; APRT, adenine phosphoribosyltransferase.

were arranged as annular formations of striated crystals or formed fan-like or irregular shapes. Within the tubular cell cytoplasm, they appeared as small single crystals with rod, needle, rhomboial or irregular shapes. In the interstitium, both single crystals as well as small to large aggregates of annular formations were identified (Figure 1D).

In all cases, DHA crystals were accompanied by multifocal acute tubular injury in non-atrophic tubules characterized by luminal ectasia, epithelial simplification, loss of the proximal tubular brush border and enlarged reactive appearing nuclei. In addition to the tubular degenerative changes, all three native biopsies showed moderate to marked irreversible tubular atrophy and interstitial fibrosis, accompanied by mild to moderate interstitial inflammation composed of lymphocytes and monocyes with rare plasma cells and neutrophils. No significant interstitial eosinophils or tubulitis were seen in any case. In case 2, DHA crystals were associated with tubular and interstitial foreign-body giant cell reaction.

On EM, the tubular cells exhibited degenerative changes including loss of brush border, cytoplasmic simplification, dilatation of endoplasmic reticulum and enlarged nucleoli. The intraluminal and intracytoplasmic crystals appeared as single or radially-oriented clusters of clear, needle or rod-shaped crystals that indent the tubular epithelium (Figure 1E). They appeared electron lucent to mildly electron dense. No tubulointerstitial or glomerular electron-dense, immune complex-type deposits were seen in any case, and the podocytes showed only minimal foot process effacement. IF, performed in all cases, confirmed the absence of immune complex-type deposits.

**Discussion**

Most reported cases of DHA disease have been from Japan, Iceland and France with only five single case reports from the USA [6–10]. The frequency of heterozygosity at the APRT locus has been estimated to be 0.4–1.1% in Caucasians, which would suggest a homozygosity rate of 1/50 000–1/100 000. Given a population of 300 million, 3000–6000 people in the USA may be at risk for developing DHA disease. The low detection rate in the USA is likely due to disease under-diagnosis considering that the percentage of asymptomatic homozygotes, estimated around 15%, is the same in all countries [13]. DHA stone disease patients are often misdiagnosed as having uric acid stones since both types of stones are radiolucent and they cannot be distinguished by routine chemical tests for stone analysis, including colorimetry reactions and thermogravimetric reactions [14]. Ultraviolet or infrared spectrophotometry is required for this distinction. The inaccurate diagnosis of uric acid stones leads to treatment with allopurinol and low-purine diet which are also effective in preventing DHA stone recurrence; therefore, patients with DHA stones are probably being successfully treated but are misdiagnosed.

DHA crystals may be seen in the urine and can provide a valuable clue to the diagnosis when recognized. Due to their rarity, however, laboratory personnel may not note them nor appreciate their significance [1]. Similarly, many pathologists are not familiar with DHA crystals, and the accurate diagnosis is typically made retrospectively, after the disease recurs in the transplant. In all four patients previously reported worldwide with recurrent DHA crystalline nephropathy in the allograft in whom the native kidney had been biopsied, the correct diagnosis was established only after transplant [3,10,11,15]. In patient 1 of our series, the correct diagnosis was delayed 56 months post-native renal biopsy, after losing her first transplant due to recurrent disease and with disease recurrence in the second transplant. In this patient, and that of Gagne et al. [11], an erroneous diagnosis of primary hyperoxaluria was initially made, with the correct diagnosis made only after the disease recurred in the transplant. Both DHA disease and primary hyperoxaluria are transmitted by autosomal recessive inheritance and may lead to nephrolithiasis and ESRD with a high rate of recurrence in the transplanted kidney. However, in contrast to primary hyperoxaluria, which is a systemic disease, DHA does not appear to be associated with any extrarenal manifestations (Table 3).

The vast majority of patients with DHA disease present with recurrent nephrolithiasis, which may lead to chronic renal failure secondary to obstructive nephropathy and recurrent urinary tract infections. Patients 1 and 2 in our se-

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**Table 3. Comparison of primary hyperoxaluria and 2,8-dihydroxyadeninuria**

<table>
<thead>
<tr>
<th>Defected enzyme</th>
<th>AGT (in type I)</th>
<th>GRHPR (in type II)</th>
<th>APRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of inheritance</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Onset of clinical symptoms</td>
<td>In childhood in most patients</td>
<td>Nephrolithiasis in children</td>
<td>In adulthood in most patients</td>
</tr>
<tr>
<td>Extrarenal manifestations</td>
<td>Cardiac conduction defects, vasculopathy, anemia, osteopathy, retinopathy</td>
<td>None known</td>
<td>None known</td>
</tr>
<tr>
<td>Gold standard diagnostic test</td>
<td>Determining AGT and GRHPR activity</td>
<td>Determining APRT activity by liver biopsy</td>
<td>Determining APRT activity in red blood cells</td>
</tr>
<tr>
<td>Treatment</td>
<td>Low oxalate diet, high fluid intake, pyridoxine, orthophosphate, combined liver–kidney transplant</td>
<td>Low-purine diet, high fluid intake, allopurinol</td>
<td>Low-purine diet, high fluid intake, allopurinol</td>
</tr>
</tbody>
</table>

AGT, alanine/glyoxalate aminotransferase; GRHPR, glyoxylate reductase/hydroxypyruvate reductase; APRT, adenine phosphoribosyltransferase.
ries developed severe renal failure due to DHA-induced crystalline nephropathy in adulthood without any history of stones. This presentation is rare with only three patients previously reported worldwide [1–3].

The diagnosis of DHA disease is confirmed by measurement of APRT activity in red blood cells. The management of patients with DHA disease due to APRT deficiency includes a low-purine diet, high fluid intake and treatment with allopurinol. This regimen prevents recurrence of nephrolithiasis and can provide preservation of renal function or even improvement of renal function if initiated early enough [1]. Unfortunately, DHA crystalline nephropathy is usually discovered late in the course of the disease, after significant tubular atrophy and interstitial fibrosis have already occurred. In patient 3 of our series, who had 60% tubular atrophy and interstitial fibrosis at presentation, treatment with allopurinol 300 mg/day did not prevent the progression to ESRD or recurrence in the allograft. Increasing the dose to 600 mg/day, however, resulted in less crystal deposition in the graft and improvement of allograft function. Patient 2, who was on 400 mg/day doses at the time of transplant, had good graft function with no evidence of recurrent disease on the 3-month post-transplant biopsy. The patient reported by Benedetto et al. was treated with doses of 10 mg/kg/day for his recurrent disease, which lead to stabilization of allograft function [10]. Based on the above albeit limited data, high doses of allopurinol (≥400 mg/day) may be needed to treat DHA crystalline nephropathy. However, in the absence of generally available laboratory measurements of DHA to guide treatment, dosing of allopurinol is largely empiric.

In two of our patients and in two patients reported by de Jong et al. [14] and Eller et al. [16], DHA disease has recurred in the first month post-transplant. The early recurrence may have been triggered by the presence of ischemic acute tubular injury as all these patients were recipients of deceased donor allografts and most of them had delayed graft function. The lower nephron mass and GFR after transplantation possibly contribute to the early disease recurrence [16].

The mechanisms by which DHA crystal deposition in the kidney leads to irreversible tubular atrophy and interstitial fibrosis are not well understood. APRT-deficient mice predictably and spontaneously develop nephrolithiasis and then renal failure. The renal histology in these APRT-deficient mice shows tubular luminal and cytoplasmic crystals, tubular necrosis and dilatation and progressive interstitial inflammation and fibrosis [17]. APRT-deficient mice were recently shown to have increased mRNA and gene expressions of MCP-1, IL-1β, CCR2 and TGF-β, as well as remarkable interstitial macrophage infiltration and increased fibroblasts [18]. Therefore, DHA crystals possibly evoke an inflammatory response through stimulating tubular epithelial cells to produce MCP-1, leading to macrophage accumulation which in turn produces TGF-β that triggers fibrosis [18]. On the other hand, the prominent acute tubular injury seen in our cases and in mice suggests a potential direct tubular toxicity by the crystals. Tubular obstruction by the intraluminal crystals may also be a factor in the development of renal damage.

Metabolic conditions that may lead to renal parenchymal deposition of crystals include hyperoxaluria (primary and secondary), hyperuricemia (including uric acid nephropathy and hypoxanthine-guanine phosphoribosyltransferase deficiency), cystinosis and DHA disease (Table 4). The differential diagnosis of crystalline nephropathy also includes drug-induced nephrotoxicity, especially indinavir, foscarinet and ciprofloxacin [19–21].

In summary, DHA disease is an under-recognized cause of irreversible renal failure with frequent recurrence in the allograft. The disease must be considered in the differential diagnosis of crystalline nephropathy, even in the absence of history of nephrolithiasis. Pathologists and nephrologists should be familiar with the characteristic brownish and birefringent tubulointerstitial crystals of this rare condition. X-ray crystallography of the crystals and measuring APRT activity in blood spots are helpful to confirm the diagnosis.

Conflict of interest statement. The results presented in this paper have not been published previously in whole or part.

<table>
<thead>
<tr>
<th>Oxalate</th>
<th>Urate</th>
<th>Cystine</th>
<th>2,8-dihydroxyadenine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birefringence</td>
<td>Yes</td>
<td>Yes in alcohol fixed tissue (usually dissolve with formalin fixation/processing)</td>
<td>Yes in alcohol fixed tissue (dissolve with formalin fixation/processing)</td>
</tr>
<tr>
<td>Staining characteristics</td>
<td>Usually transparent (deep blue if admixed with calcium)</td>
<td>Transparent or pale blue (deep blue if admixed with calcium)</td>
<td>Difficult to see (transparent)</td>
</tr>
<tr>
<td>Shapes and arrangements</td>
<td>Rhomboid shapes often with cluster or rosette-like arrangement</td>
<td>Elongated, rectangular or amorphous shapes, usually in large collections</td>
<td>Rectangular or needle shapes, present singly or in small collections</td>
</tr>
<tr>
<td>Distribution</td>
<td>Mainly in tubular lumina and cytoplasm, sometimes in the interstitium (more abundant in the cortex)</td>
<td>Mainly in collecting ducts lumina and interstitium in the medulla (not in tubular cells)</td>
<td>Mainly in interstitium, sometimes in tubular cells and podocytes</td>
</tr>
<tr>
<td>Giant cell reaction to crystals</td>
<td>Sometimes in interstitium</td>
<td>Common in tubules and interstitium</td>
<td>Multinucleated podocytes</td>
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</table>

Table 4. Pathologic differential diagnosis of crystalline nephropathy caused by metabolic diseases
DHA crystalline nephropathy 1915

References


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