Recurrent primary hyperoxaluria type 2 leads to early post-transplant renal function loss: A case report

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Abstract. Primary hyperoxaluria type 2 is a rare autosomal recessive disorder caused by glyoxylate reductase/hydroxypyruvate reductase deficiency and characterized by recurrent episodes of nephrolithiasis and nephrocalcinosis. Herein, we describe a case of primary hyperoxaluria type 2 in a 33-year-old man who failed to respond to conventional therapies; thus renal transplantation was performed. This case demonstrated that, although primary hyperoxaluria type 2 is rare, hyperoxaluria should be suspected and blood oxalate and stone component be examined in patients with recurrent episodes of nephrolithiasis, particularly in those who are unresponsive to conventional therapies. Combined liver-kidney transplant may be required as kidney transplant alone is not likely to be successful.

Introduction

Primary hyperoxaluria is a rare autosomal recessive disorder of glyoxylate metabolism in which specific deficiencies of hepatic enzymes cause excessive oxalate production (1). Primary hyperoxaluria accounts for ~10% of pediatric patients with nephrocalcinosis (2) and 2% of patients undergoing renal replacement therapy (3). There are three known types of primary hyperoxaluria; however, the mechanism involved in manifestation are unknown (4). These known types of primary hyperoxaluria are caused by deficiencies of AGXT, GRHPR and HOGA1, which encode alanine, glyoxylate aminotransferase, reductase/hydroxypyruvate reductase and hydroxyoxoglutarate aldolase, respectively (5). GPHPR, which is defective in primary hyperoxaluria type 2, catalyzes the conversion of hydroxypyruvate to d-glycerate (6). Primary hyperoxaluria type 2 is characterized by recurrent episodes of nephrolithiasis and nephrocalcinosis (7). In some cases, primary hyperoxaluria type 2 may result in end-stage renal disease; however, the prevalence of this is low (8). Conservative measures, including adequate hydration and oral citrate supplementation, are essential to preserve renal function and prevent nephrolithiasis for patients with early hyperoxaluria (4). Molecular targeted therapy is still currently being explored in cell systems and animal models, but has not been fully investigated in humans (9).

The present study described a case of primary hyperoxaluria type 2 with multiple bouts of nephrolithiasis resistant to lithotripsy and conservative therapy, which eventually progressed to chronic renal failure requiring kidney transplantation.

Case report

A 33-year old man was admitted to our hospital (Transplant Center, First Hospital of Jilin University, Changchun, China) in November 2014 because of chronic renal failure for 2 years. He was diagnosed with nephrolithiasis in March 2004, at which time a single stone in the right kidney was identified and removed by lithotripsy. Multiple renal calculi in bilateral kidneys were identified in June, 2009 and managed via lithotripsy and the patient received *Quercus salicina* extract capsules (450 mg; three times daily for 3 weeks). However, bilateral renal calculi recurred 2 months after the lithotripsy, and the patient underwent multiple lithotripsies thereafter. In November 2014, the patient developed dizziness and nausea and his serum creatine levels were measured as 2,500 mol/l (normal reference values, 44 to 115 μmol/l). The patient was diagnosed with chronic renal failure and underwent maintenance dialysis.

The patient received an allogeneic renal transplant in December 2014 at our Transplant Center. The Transplant was performed in accordance with the approved guidelines by the Ethics Committee of Transplant Center, First Hospital of Jilin University and the patient provided signed and informed consent. Urine was passed 30 sec following the restoration of blood flow. The patient was administered intravenous anti-human T thymocyte rabbit immunoglobulin (100 mg once daily for 6 days; Fresenius SE & Co. KGaA, Bad Homburg, Germany) and methylprednisolone (500 mg once daily for 3 days; Pfizer, Inc., Reno, NV, USA) and oral tacrolimus (0.1 mg/kg/day; Astellas Ireland Co., Ltd., Kerry,

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Ireland), mycophenolate (540 mg twice daily; Novartis, Basel, Switzerland), and prednisone (120 mg; Xianju Pharmaceuticals Co., Ltd., Hangzhou, Zhejiang, China) on post-transplant day 3 when intravenous methylprednisolone was completed. Oral prednisone was then reduced 20 mg/day until the dosage was 20 mg. The 24-h urine volume was recorded as 11,350 ml on day 1 post-surgery. Serum creatine (normal reference values, 44 to 115 mol/l) decreased to 198 μmol/l on day 5 post-surgery and rose to 214 mol/l on day 6 post-surgery. The patient's blood concentration of tacrolimus was 9.1 ng/ml on day 8 post-surgery and a renal graft biopsy was performed. Biopsy tissues were fixed in 10% formaldehyde at room temperature for 4 h and sectioned at a thickness of 2 μm. Following standard protocols, sections were processed and stained with hematoxylin and eosin (H&E) staining. Briefly, the sections were stained in hematoxylin at room temperature for 10 min, followed by staining in 1% eosin solution at room temperature for 3 min, and subsequently washed with distilled water. The sections were then placed into Schiff's reagent and incubated for 30 min at room temperature. The slides were observed under light microscope (BX5; Olympus Corp., Tokyo, Japan) and mild acute nephrotoxicity was observed (Fig. 1). The dose of tacrolimus was reduced to 0.075 mg/kg/day; however, no decline in serum creatine was observed. On day 15 post-surgery, serum creatine levels gradually increased to 291 μmol/l. A renal graft ultrasound found hyperechocity of the renal cortex, and the transplanted kidney was 11.5x5.6x5.5 cm in size, with a renal artery resistive index of 0.65 (normal range, 0.6-0.8). The tacrolimus dose was further reduced to 0.05 mg/kg/day and 1.0 mg/day sirolimus (Pfizer Ireland Pharmaceuticals, Kildare, Ireland) was added. Blood rapamycin content was 6.1 ng/ml, tacrolimus 5.1 ng/ml, and the area under the curve (AUC) of mycophenolate mofetil was 33.38.

ELISA-plasma activity test for human leukocyte agent class II was performed using an ELISA kit (LATM10X5, LOT001; One Lambda; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions and the results were negative. There was no noticeable improvement in renal function, and serum creatine was 290 μmol/l. A renal biopsy was performed on day 26 post-surgery and the sections were processed and stained with periodic acid-Schiff (PAS) stain following standard protocol. The sections were immersed in 1% periodic acid at room temperature for 15 min, and subsequently washed with distilled water before the sections were placed into Schiff's reagent and incubated 30 min at room temperature. The slides were observed under light microscope (BX51; Olympus Corp), which revealed borderline lesions of the renal graft and tubulitis (1+) that was accompanied by a small amount of crystal deposition within the renal tubules (Fig. 2). Primary hyperoxaluria was considered. The patient was administered with sodium bicarbonate tablets (1.0 g 3 times daily), oral vitamin B6 (100 mg twice daily), and *Quercus salicina* extract capsules (450 mg 3 times daily) for discharging stones; however, no improvement was seen. Serum creatine rose to 376 μmol/l, and a protocol biopsy on day 37 post-surgery revealed borderline lesions of the renal graft, tubulitis (1+), and crystal deposition within the renal tubules, which was worse than that on day 26 (Fig. 3).

A peripheral blood sample was taken from the patient and genomic DNA was extracted using the TIANamp Blood DNA kit (DP348-02 DP348-03; Tiangen Biotech, Co., Ltd., Beijing, China) as instructed by the manufacturer. Primers covering all coding regions and flanking introns of the *Ph1* and *Ph2*, *AGXT* and *GRHPR* genes were designed and synthesized (Qingwei, Wuhan, China) according to the gene sequences from Ensembl (http://asia.ensembl.org/index.html) via NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast) (Primer sequences are available upon request). The gene sequences were amplified by polymerase chain reaction (PCR) (2X PCR MasterMix polymerase; Tiangen Biotech, Co., Ltd., Beijing, China). The PCR conditions were as follows: 95˚C for 5 min followed by 95˚C for 30 sec; 56˚C for 30 sec; 72˚C for 30 sec

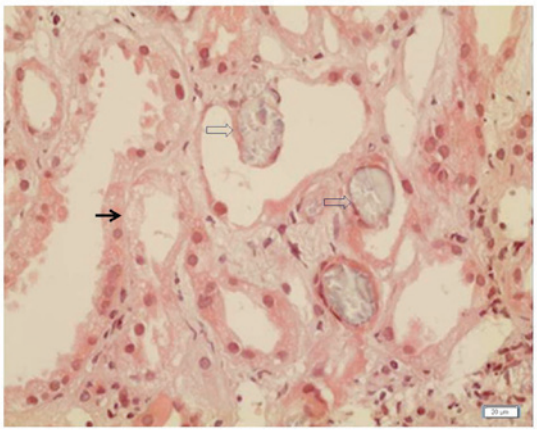

Figure 1. Results of a renal biopsy in a 33-year old allogeneic renal transplant patient on day 8 post-surgery (hematoxylin and eosin stain; magnification, x40; scale bar, 20 μm). Glomerulosclerosis was observed in 2/9 glomeruli. There was mild proliferation of mesangial cells and matrix. Mild vacuolation was observed in renal tubule epithelial cells (indicated by the arrow), crystal-like substances were occasionally seen in the renal tubule epithelial cells (indicated by hollow arrow), and the interstitia had multifocal edema. No apparent infiltration of inflammatory cells and fibrosis was observed. Mild thickening of the arterioles and hyaline changes in the microvascular wall were noted. Based on these observations, acute nephrotoxicity due to immunosuppressive drugs was considered.

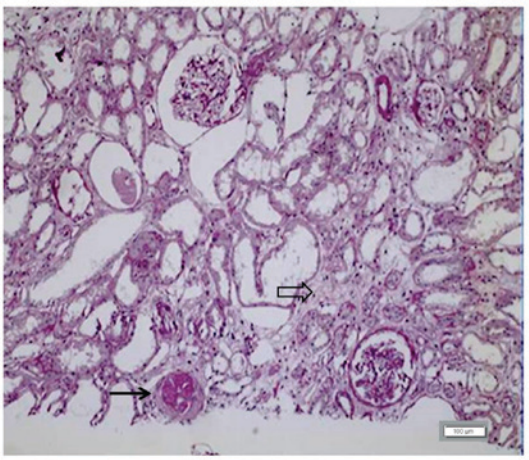

Figure 2. Results of a protocol renal biopsy in a 33-year old allogeneic renal transplant patient on day 26 post-surgery (PAS stain; magnification, x10; scale bar, 100 μm). Borderline lesions of the renal graft, partial glomerulosclerosis (indicated by arrow) and renal tubulitis were observed. A small amount of needle-like crystal deposition in the renal tubule epithelia and multifocal edema (indicated by hollow arrow) in the interstitia were also noted. The biopsy revealed infiltration by macrophages and mild fibrosis.

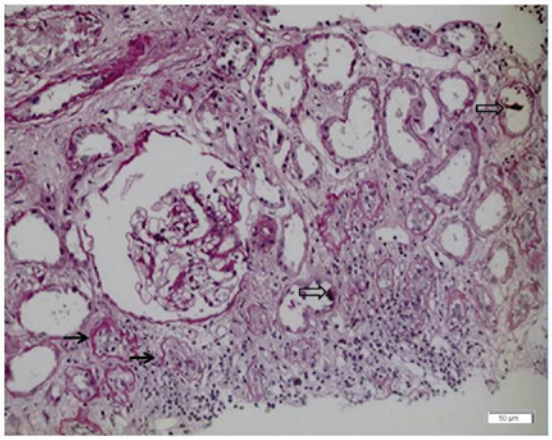

Figure 3. Results of a protocol renal biopsy in a 33-year old allogeneic renal transplant patient on day 37 post-surgery (PAS stain; magnification, x20; scale bar, 50 μm). Borderline lesions of the renal graft, tubulitis (1+) (indicated by arrow), and crystal deposition within the renal parenchyma were observed (indicated by hollow arrow).

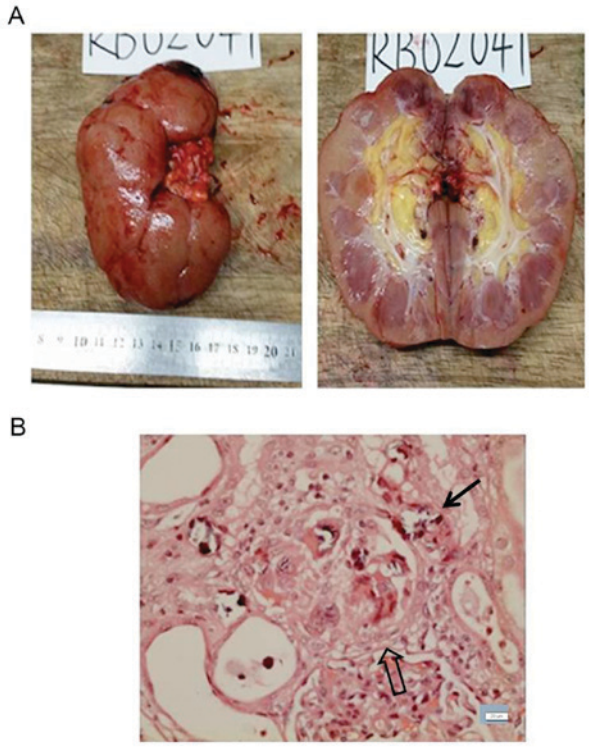

Figure 4. Renal pathology of the excised renal graft from a 33-year-old allogenic renal transplant patient. (A) Gross appearance of the renal graft; the kidney measured 13x7x4.5 cm and the border between the cortex and medulla was distinct. The cortex was 0.6 cm in thickness and several gray white stones were observed in the renal pelvis. (B) Light microscopy (PAS stain; magnification, x40; scale bar, 20 μm) revealed partial glomerulosclerosis and accumulation of blue-stained neutrophilic crystals, partially obstructing the renal tubules (indicated by arrow) and depositing in the renal interstitia. Focal sclerosis and stones were also observed (indicated by hollow arrow).

for a total of 35 cycles and an additional incubation at 72˚C for 10 min. PCR products were purified and sent to Grandomics Biosciences Co., Ltd. (Beijing, China) for sequencing analysis. The sequencing results showed that the patient was negative for *Ph1* and *Ph2*. Furthermore, no mutations were detected in the *AGXT* exon coding region. However, a homozygous mutation was detected in the *GRHPR* gene, and a heterozygous mutation in the gene was detected in the patient's mother.

The patient's urine volume gradually declined and dialysis was maintained at day 42 post-transplant. Due to repeated episodes of fever, nausea, fatigue, and viral infections, the patient requested that the renal transplant be removed. In February 2015 at day 54 post-transplant, the patient underwent a second surgery under general anesthesia. An incision was made in the lateral border of the rectus abdominis and the graft renal artery and vein were located, ligated and excised. The ureter was also ligated and excised and the renal graft was removed. Renal pathology revealed interstitial injury due to crystal deposition within the renal tubules, with renal pelvis stones (Fig. 4). Chemical analysis found that the kidney stones were calcium oxalate. The patient received post-transplant hemodialysis and the date of the last follow-up visit was October 2015.

Discussion

Primary hyperoxaluria, a rare autosomal recessive disorder, is characterized by hyperoxaluria accompanied by early and recurrent episodes of nephrolithiasis, which eventually causes renal injury as a result of calcium oxalate crystal deposition (7). Due to its rarity, physicians are typically unfamiliar with the condition, resulting in delayed or missed diagnosis (10). The present patient suffered multiple bouts of nephrolithiasis recalcitrant to lithotripsy and conservative therapy, which eventually progressed to chronic renal failure, leading to allograft transplantation. Undiagnosed primary hyperoxaluria also contributed to the eventual failure of the renal graft. Two protocol biopsies failed to reveal crystal deposition in the renal tubules. Primary hyperoxaluria type 2 was only diagnosed following the detection of crystal depositions in the renal tubules and molecular genetic testing.

There are three types of primary hyperoxaluria. Type 1 is caused by a deficiency of the liver peroxisomal enzyme alanine-glyoxylate-aminotransferase (AGT), which catalyzes the conversion of glyoxylate to glycine (11). Type 2 is caused by a GPHPR deficiency, which catalyzes the conversion of hydroxypyruvate to d-glycerate (6). An enzyme deficiency in type 3 has not been unambiguously identified and mutations in the *DHDPSL* gene have been reported (12,13). Primary hyperoxaluria is typically associated with early onset of symptoms, presenting in patients between 1 and 25 years of age (14). In the present case, the patient was diagnosed in adulthood; however, he suffered symptoms early in life. The findings from 24-h urine oxalate, urinary oxalate-to-creatinine molar ratio and plasma oxalate suggested primary hyperoxaluria type 1 (15), as did hepatic AGT activity (16); however, the final diagnosis was dependent on molecular genetic testing (12,17). As primary hyperoxaluria 2 exhibits a similar phenotype to type 1, its ultimate diagnosis relies on the conclusion of molecular genetic testing (13). When primary hyperoxaluria is suspected gene testing for type 1 should be performed first as it is more common (18).

Primary hyperoxaluria is managed by ensuring that the patient has adequate fluid intake, is given urinary inhibitors of calcium oxalate crystallization and vitamin B6, and undergoes

routine dialysis (4). In this patient, these conservative measures failed to control unrelenting bouts of nephrolithiasis and nephrocalcinosis. Kidney transplantation alone has been used to treat primary hyperoxaluria with varied results (19,20). In cases of hyperoxaluria type 1, although the renal graft is able to function, excessive oxalate production occurs in the liver, which continues to deposit calcium oxalate in the renal parenchyma and tubules (21). Therefore, renal transplantation is not recommended for type 1 patients (21). In the present case, kidney grafting was performed; however, it partially failed due to recurrent nephrolithiasis. Renal function in the patient deteriorated soon after renal transplantation, and primary hyperoxaluria was diagnosed ~4 weeks post-surgery when crystal deposition in the renal tubules was demonstrated. This suggests that calcium oxalate deposition occurs early after renal transplant, jeopardizing renal function recovery and indicating that renal transplant may not be a viable treatment option for type 2 patients.

In conclusion, although primary hyperoxaluria type 2 is rare, it should be considered in patients with recurrent episodes of nephrolithiasis, particularly those who are unresponsive to conventional therapies. Blood oxalate and stone components should be examined in such patients. Combined liver-kidney transplant may be required, as kidney transplant alone is unlikely to be successful.

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Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors' contributions

SL, BG and HZ mainly participated in the literature search, study design, writing and critical revision. GW, WW, XL, SW, JY and YF mainly participated in data collection, data analysis and data interpretation. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was carried out in accordance with the approved guidelines by the Ethics Committee of First Hospital of Jilin University and the patients provided their signed and informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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