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Case Series

Chronic Liver Disease in Patients with Prolidase Deficiency: A Case Series

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Keywords
Prolidase deficiency · Liver disease · Hepatic involvement

Abstract
Introduction: Prolidase deficiency is a rare autosomal recessive disorder caused by variants in the *PEPD* gene. Patients usually have multi-organ involvement and a wide range of clinical features including recurrent skin ulcers, dysmorphic facial features, recurrent infections, intellectual disability, and splenomegaly. Studies have shown that patients with prolidase deficiency may have hepatic manifestations including hepatomegaly and abnormal liver enzymes. However, there is no detailed description of liver disease in this patient population. ***Case Presentation:*** Here, we present 3 patients with prolidase deficiency with varying extents of hepatic involvement. ***Conclusion:*** Prolidase deficiency patients with liver disease should be followed up long term to understand more about the pathophysiology and the impact of liver disease on long-term outcomes.

Introduction

Prolidase is the only known metalloproteinase capable of cleaving imidopeptides containing C-terminal proline or hydroxyproline and helps in the release of proline or hydroxyproline [1]. Prolidase is necessary for remodeling of extracellular matrix by recycling

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proline for collagen synthesis [2]. The *PEPD* gene located on the long arm of chromosome 19 encodes the prolidase enzyme and pathological variants in *PEPD* results in prolidase deficiency [3]. Patients can present with wide array of clinical features including chronic recurrent skin ulcers, recurrent infections, dysmorphic facial features, variable intellectual disability, developmental delay, and splenomegaly [4]. A systematic review of published cases of prolidase deficiency by F. Rossignol et al. showed that 13.5% had hepatomegaly, 6.7% had elevated transaminase levels and 4.5% had liver disease out of 178 patients [5]. There is as yet no detailed description of liver involvement in the literature. Here, we present 3 patients with prolidase deficiency with varying stages of liver involvement and liver disease progression.

Case Presentation

Patient 1

A 35-year-old female with prolidase deficiency with multi-organ involvement including recurrent lower extremity ulcers, recurrent epistaxis, protein C and S deficiency, telangiectasias, pulmonary arteriovenous malformations requiring coiling, and developmental delay. As per patients' mother, patient was found to have elevated transaminase levels (exact values unavailable) at the age of 10 years and at that time had extensive workup including a liver biopsy all of which was unrevealing. At age 12, she was found to have hepatosplenomegaly (data regarding liver biopsy and liver size is unavailable) with persistent thrombocytopenia of unclear etiology for which she eventually required a splenectomy resulting in improvement of thrombocytopenia. A trans-jugular liver biopsy performed at that time showed a hepatic venous pressure gradient of 10 mm Hg but normal liver histology. Since the age of 2 years, she suffered from consequences of prolidase deficiency but was only diagnosed at age of 24 years. During her first visit to our institution, she complained of fatigue and generalized itching. She has no history of alcohol, intravenous drug, or herbal medication use. Her parents are from Iraq and are first cousins.

During initial evaluation, her vital signs were normal, her BMI was 23 kg/m^2, physical exam was notable for telangiectasias in bilateral palms, flattened nasolabial folds, low hairline, flattened nasal bridge, and healed surgical scar in left hypogastric region. There was a 2 cm deep ulceration in the left heel with granulation tissue without oozing or discharge. Complete blood count and basic metabolic panel were unremarkable. Platelet count was 265 × 10^9/L. Remaining pertinent laboratory studies included alkaline phosphatase (ALP) of 293 U/L, alanine aminotransferase (ALT) of 53 U/L, aspartate aminotransferase (AST) of 91 U/L (Fig. 1), total bilirubin 0.8 mg/dL with direct bilirubin 0.3 mg/dL, prothrombin time 16.7 s, INR 1.33, albumin 2.9 g/dL, and ferritin 199 μg/L. Viral hepatitis and autoimmune hepatitis panels were negative. Ultrasound of the abdomen showed that the liver was normal in size (12.2 cm) but that the left lobe was prominent. It had a smooth contour and normal texture. Portal and hepatic veins were patent with normal diameter and flow direction. Magnetic resonance imaging of the liver (Fig. 2) showed atrophic right lobe, enlarged lateral segment of left lobe, and caudate lobe suggestive of chronic liver disease.

At age 24, she had a trans-jugular liver biopsy (Fig. 3) which showed mild predominantly lobular hepatitis associated with focal consolidation with hemorrhage, sinusoidal dilatation and mild perisinusoidal and periportal fibrosis, copper accumulation suggestive of chronic cholestasis. Measurements of hepatic venous portal gradient (HVPG) in the left and right lobes of the liver showed hepatic wedge pressures of 6–7 mm Hg indicating mildly elevated sinusoidal pressures. Based on liver histology findings and elevated ALP, she was started on cholestyramine, but her itching persisted, and she developed constipation. So, she was

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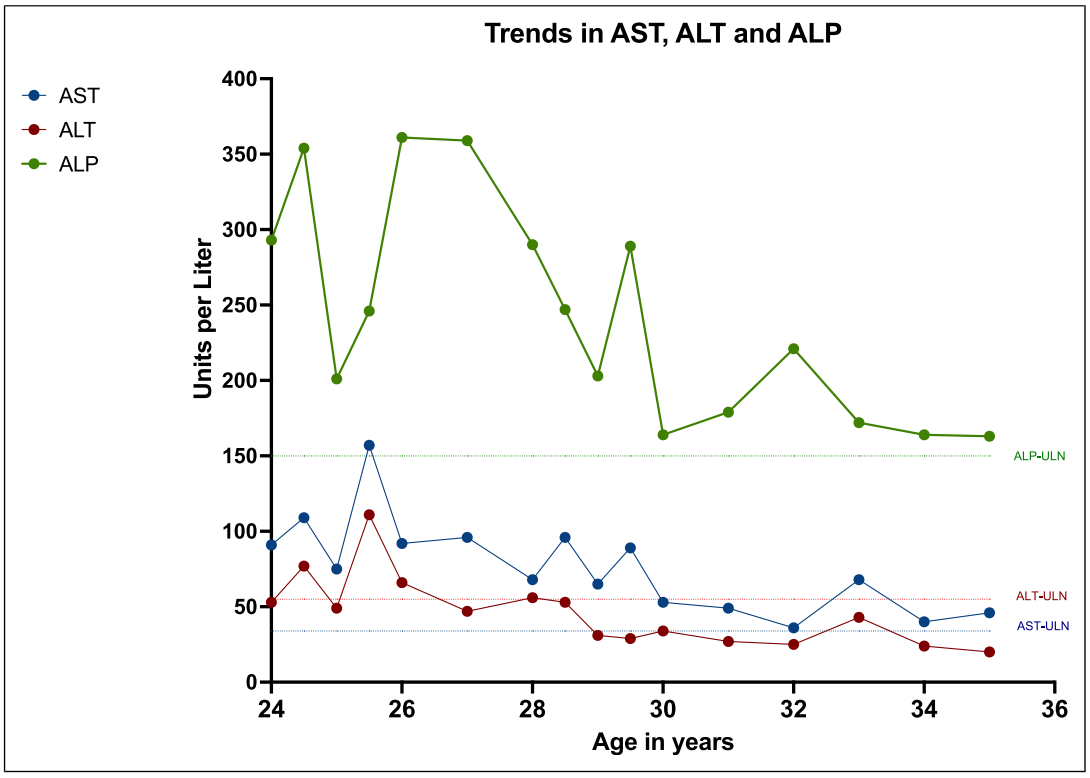

Fig. 1. Trend in liver enzymes over time. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ULN, upper limit of normal.

switched to rifampin 300 mg once a day with improvement in itching and subsequent addition of ursodiol 300 mg twice daily resulted in resolution of symptoms. Her liver disease remained stable during routine follow-up for the next few years.

At the age of 32, follow-up ultrasound showed reversal of portal vein flow and irregular liver contour compatible with cirrhosis. A repeat trans-jugular liver biopsy was done which showed chronic cholestatic liver disease with mild inflammation and portal venopathy with bridging fibrosis. Her corrected hepatic venous wedge pressure measuring 6 mm Hg suggesting minimally elevated portal venous pressure. Her MELD-Na score was 12, Child-Pugh class B with a score of 7. This has remained stable over the last 1 year. She is currently being evaluated for liver transplantation.

Patient 2

23-year-old Amish male diagnosed with prolidase deficiency at the age of 3 years, with a history of chronic skin ulcers, recurrent skin infections, recurrent ear and sinopulmonary infections, hypotonia, splenomegaly (found when he was 2 years old), elevated IgE, and thrombocytopenia. During his initial visit in this hospital at the age of 23 years, he was evaluated by the hepatology team for portal vein dilation, thrombocytopenia, and splenomegaly. He denied any symptoms related to liver disease. He had extensive evaluation by the hematology team and was not found to have any hematologic reason for thrombocytopenia and splenomegaly. He has been on minocycline 100 mg twice daily for prophylaxis since he was 16 year old. He denied any alcohol or herbal medication use. His vital signs were normal, but BMI was 36.2 kg/m^2. Physical examination was remarkable for hypertelorism, saddle shaped nose, and digital clubbing. Abdominal examination was

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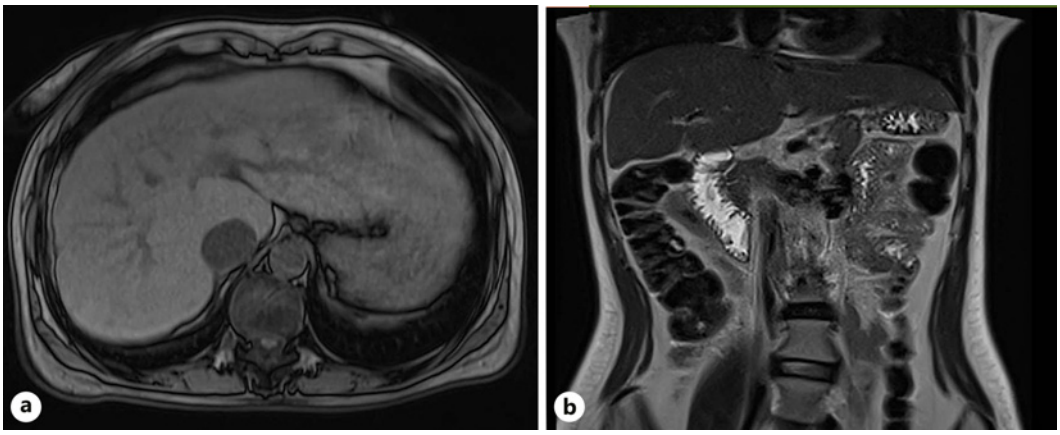

Fig. 2. Magnetic resonance imaging of the liver, (**a**) axial and (**b**) coronal view showing atrophic right lobe, enlarged left lateral segment.

remarkable for palpable spleen 4 cm below the left costal margin and non-tender, non-erythematous edema of penile shaft and scrotum.

Laboratory studies revealed a platelet count of 83 × 10^9/L, prothrombin time of 14.4 s, INR 1.10, albumin 3.9 g/dL, ALP 62 U/L, ALT 16 U/L, AST 20 U/L, total bilirubin 0.6 mg/dL, gamma glutamyl transferase 19 U/L, ferritin 1,860 µg/L, transferrin saturation 13%, IgA 5 ng/dL, and IgE 197 IU/mL. Ultrasound of scrotum/testicle was suggestive of bilateral testicular lymphedema, without evidence of hydrocele or cellulitis. He had computed tomography of the abdomen done which showed an enlarged caudate lobe, otherwise normal liver, portal vein diameter of 1.7 cm and spleen of 20.9 cm. These findings were concerning for non-cirrhotic portal hypertension, and he was advised to get trans-jugular liver biopsy with portal pressure measurements which the patient is currently contemplating.

Patient 3

A 6-year-old male with prolidase deficiency was diagnosed at the age of 3 years with multisystem involvement including oropharyngeal dysphagia requiring nasogastric tube placement for feeding during infancy, splenomegaly (found at the age of 3 years), oligoarticular juvenile idiopathic arthritis treated with methotrexate administered subcutaneously once weekly since the age of 5, recurrent pneumonia and thrombocytopenia. He was evaluated by the hepatology team at the age of 5 years for persistent elevation in transaminases since he was 2 years old. Prior to evaluation, he had extensive evaluation at an outside hospital and workup was unrevealing. During the visit, he complained of retching secondary to oropharyngeal dysphagia which has been present since he was an infant. He denied any other complaints. He continues to be on tube feeds but tolerates minimal solid food orally. Other than methotrexate, he is on pimecrolimus, clonidine, and hydroxyzine. There is no family history of liver disease or cirrhosis.

His vital signs were normal, with BMI of 14.7 kg/m^2 (25th percentile). His physical examination revealed hypertelorism, flat nasal bridge, submucosal cleft palate, abdominal exam revealed enlarged spleen palpable 2–3 cm below the costal margin. His laboratory workup revealed platelet count of 101 × 10^9/L, prothrombin time 14.6 s, INR 1.12, albumin 4.3 g/dL, ALP 200 U/L, ALT 40 U/L, AST 82 U/L, gamma glutamyl transferase 22 U/L, total bilirubin 0.7 mg/dL, direct bilirubin 0.2 mg/dL, ferritin 922 µg/L, iron 60 µg/dL, transferrin percentage saturation 14%, and creatine kinase was normal. His IgG, IgA, and IgE levels were

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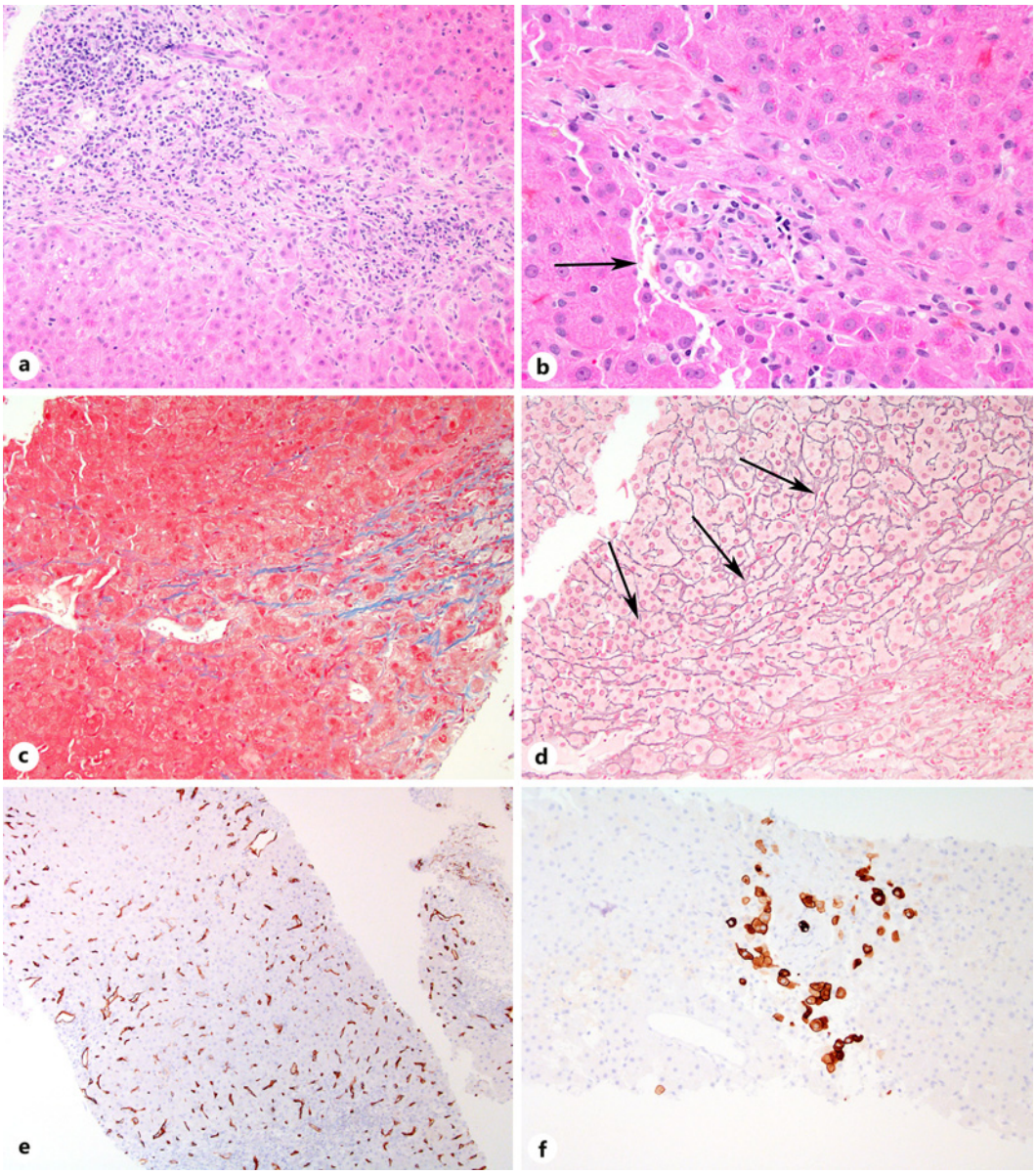

Fig. 3. Hepatic changes in prolidase deficiency. **a** Inflamed portal area with pale periportal hepatocytes indicative of chronic cholestasis (H&E, ×200). **b** Small portal area with a slit-like portal vein (arrow) (H&E, ×400). **c** Perisinusoidal fibrosis was present near central veins (Masson trichrome, ×200). **d** Regenerative nodularity, demonstrated by curved sinusoidal spaces (arrows). (Reticulin, ×200). **e** Abnormal staining of sinusoidal endothelial cells for CD34 (anti-CD34, ×100). **f** Periportal hepatocytes expressing keratin 7 consistent with chronic cholestasis (anti-keratin 7, ×200).

within normal range. Ultrasonography of the abdomen showed a liver of 12.8 cm with normal echogenicity, portal vein diameter, and flow. The spleen was 13.2 × 12.2 cm. Ultrasonography performed in the past was compared and showed a spleen size of 9.6 cm (at age 2 years) and 12.2 cm (at age 3). He currently has annual hepatology surveillance.

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Discussion

The overall estimated incidence of prolidase deficiency is 1–2 cases per million births. It has been described as more frequent in the Druze and Arab minority in Israel [6, 7]. Prolidase is an enzyme which is essential for collagen turnover and its deficiency results in poor wound healing. Most common clinical features include facial dysmorphism present in about 93%, followed by recurrent infection in 76% and splenomegaly in about 72% of patients [8]. All patients presented here suffered from the most classical features of prolidase deficiency including facial dysmorphism, recurrent nonhealing ulcers, and splenomegaly diagnosed during childhood. A systematic review showed that 13.5% had hepatomegaly, 6.7% had elevated transaminase levels, and 4.5% had liver disease [5]. However, there is no description of the exact nature of, nor outcomes of liver disease in patients with prolidase deficiency. All the patients presented here had thrombocytopenia with 1 patient having improvement postsplenectomy, 2 out of 3 patients had persistent elevation in transaminase levels (Table 1), and 1 patient had progression from nodular regenerative hyperplasia to bridging fibrosis on follow-up. All the patients had extensive workup and other causes of chronic liver disease were excluded. The underlying pathophysiology for liver disease is not clearly understood. It is intriguing to speculate whether the observed thrombocytopenia is due to portal hypertension, particularly non-cirrhotic portal hypertension as may have been present in patient 1 above, where there was likely a component of presinusoidal liver disease.

Mayra et al. [9] hypothesized that plasma prolidase activity monitoring can be useful to evaluate fibrotic processes in patients with chronic liver disease. In a study involving subjects with alcoholic liver disease, it was found that those with alcoholic hepatitis have higher plasma prolidase activity than patients with stable cirrhosis [10]. Patients with nonalcoholic steatohepatitis have been shown to have higher plasma prolidase enzyme activity in comparison to healthy controls and those with simple steatosis [11, 12]. Even in chronic hepatitis B patients, it has been shown that prolidase levels are higher compared to healthy controls [13]. Studies using rat model of cirrhotic liver fibrosis by Abraham et al. [14] suggested that serum prolidase activity may be higher in the early stages of fibrosis. This was later confirmed in studies involving human subjects which showed that serum prolidase levels were significantly higher in patients with hepatocellular carcinoma compared to healthy patients [15]. These results indicate that there might be a role for serum prolidase in the development of different forms of chronic liver disease. It is therefore interesting to note that patients with prolidase deficiency develop chronic liver disease and even progress to bridging fibrosis.

The underlying pathophysiology of common clinical manifestations in prolidase deficiency remains unclear. One proposed mechanism involves increased imidodipeptide accumulation, impacting cellular functions [16]. Another potential factor is the absence of the prolidase enzyme, leading to reduced hypoxia-inducible factor 1 alpha (HIF-1α) and compromised angiogenesis signaling [17]. Interestingly, HIF-1α plays a complex role in chronic liver diseases like alcoholic and nonalcoholic fatty liver disease, exhibiting both protective and detrimental effects [18]. However, its specific role in prolidase deficiency remains unclear, necessitating further exploration. Additionally, nuclear factor-κB (NF-κB) expression has shown to be upregulated with inhibition of prolidase enzyme [19]. NF-κB, a key transcriptional regulator of inflammation has shown to play a key role in hepatic inflammation and fibrosis [20]. This upregulated NF-κB may contribute to liver disease in prolidase deficiency. A comprehensive evaluation is needed to unravel the connections between prolidase deficiency, HIF-1α, NF-κB, and their impact on liver disease seen in these patients.

In conclusion, patients with prolidase deficiency can develop advanced liver disease and should be followed up closely with a hepatologist. Future efforts should concentrate on understanding more about the pathophysiology and the impact of liver disease on long-term

Table 1. Comparison of laboratory results and *PEPD* gene mutation

Parameters	Patient 1	Patient 2	Patient 3
Initial symptoms	Recurrent nonhealing ulcers in lower extremity	Recurrent infection and nonhealing ulcers in lower extremity	Failure to thrive
Reason for suspecting prolidase deficiency	Clinical suspicion	Clinical suspicion	No clinical suspicion, however, found upon investigation
Albumin, g/dL (reference range)	2.1–3.3 (3.8–4.7)	3.9–4.3 (3.8–4.7)	4.3 (3.8–4.7)
Aspartate aminotransferase, U/L (reference range)	36–157 (5–34)	24–42 (5–34)	72–101 (26–55)
ALT, U/L (reference range)	15–111 (0–55)	9–17 (0–55)	26–51 (11–30)
ALP, U/L (reference range)	145–497 (40–150)	62–162 (40–150)	170–200 (156–369)
Total bilirubin, mg/dL (reference range)	0.3–1 (0.2–1.2)	0.6 (0.2–1.2)	0.7 (0.1–0.4)
Direct bilirubin, mg/dL (reference range)	0.1–0.5 (0.0–0.3)	0.3 (0.0–0.3)	0.2 (0.1–0.2)
Gamma-glutamyl transferase, U/L (reference range)	91–342 (9–36)	19 (12–64)	19–22 (6–16)
Platelet count, $\times 10^9$/L (reference range)	116–477 (173–369)	77–113 (161–347)	101–140 (206–369)
Mean platelet volume, fL (reference range)	10–12.1 (9.4–12.4)	9.8–10.2 (9.4–12.4)	10.6–11.8 (9.2–11.4)
Type of sequencing	Targeted single-gene sequencing	Targeted single-gene sequencing	Exome sequencing
PEPD gene Zygosity, DNA change (protein change) (NM_000285.4)	Homozygous c.549-1G>A, splice variant	Homozygous c.793C>T; p.Arg265 Ter	Heterozygous c.977G>A,p. Trp326Ter and heterozygous c.1244T>A; p. Ile415Asn

outcomes. The CARE Checklist has been completed by the authors for this case series, attached as supplementary material (for all online suppl. material, see https://doi.org/10.1159/000536117).

Statement of Ethics

This study protocol was reviewed and approved by National Institutes of Health – Institutional Review Board, approval number 07I0033. Written informed consent was obtained from the patient 1 and 2 for publication of the details of their

medical case and any accompanying images. Written informed consent was obtained from the parent of the patient 3 for publication of the details of their medical case and any accompanying images.

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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Author Contributions

Conception and design: H. Gopalakrishna, and T. Heller. Administrative support: H. Gopalakrishna, T. Heller, D.E. Kleiner, and A.F. Freeman. Provision of study materials or patients: H. Gopalakrishna, D.E. Kleiner, A.F. Freeman, T. Heller, Hari S. Conjeevaram, Bilal Asif, and Anjali Rai. Collection and assembly of data: H. Gopalakrishna, B. Asif, A. Rai, A.F. Freeman, T. Heller, and M. Mironova. Manuscript writing: H. Gopalakrishna, B. Asif, A. Rai, A.F. Freeman, and T. Heller. Final approval of the manuscript: H. Gopalakrishna, B. Asif, A. Rai, H.S. Conjeevaram, M. Mironova, D.E. Kleiner, A.F. Freeman, and T. Heller.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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