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Lysinuric protein intolerance: Pearls to detect this otherwise easily missed diagnosis

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Abstract.
BACKGROUND: Lysinuric protein intolerance (LPI) is a rare autosomal recessive disorder characterized by deficient membrane transport of cationic amino acids. It is caused by pathogenic variants in SLC7A7, resulting in impairment of intestinal import and renal proximal tubule loss of the affected amino acids. LPI typically presents with gastrointestinal symptoms, such as vomiting, diarrhea, and failure to thrive.
CASE REPORT: A 4-year-old African-American boy presented with multiple respiratory tract infections, weight loss in the setting of chronic diarrhea and worsening abdominal distention, and multiple episodes of rectal prolapse. Development was unaffected. Laboratory examination demonstrated mild anemia, hypokalemia and hypoalbuminemia, transaminitis, and normal ammonia. Initial urine amino acid analysis did not show major elevations of lysine and ornithine, often lower than expected in the setting of malnutrition. Upon initiation of total parenteral nutrition (TPN), his urine amino acids showed a characteristic profile of dibasic aminoaciduria.
CONCLUSIONS: Failure to thrive, chronic diarrhea, and hepatomegaly should raise suspicion for LPI. Urine amino acids can be normal in this condition in the setting of malnutrition, a common complication of the disease. Additionally, it has been previously shown that the plasma arginine and ornithine concentration is higher in LPI subjects.

Keywords: Dibasic amino aciduria II, hyperdibasic aminoaciduria, LPI - lysinuric protein intolerance, SLC7A7

1. Introduction

Lysinuric protein intolerance (LPI; OMIM #222700) is an autosomal recessive disorder characterized by deficient membrane transport of cationic amino acids lysine, ornithine and arginine. In most cases, it is caused by pathogenic variants in SLC7A7 on chromosome 14 at locus 14q11.2 [2, 21], although variants cannot be identified in this gene in 5–8% of cases [12, 19]. SLC7A7 encodes the $y+$L amino acid transporter-1 ($y+$LAT-1), which is responsible for the transport of dibasic amino acids at the basolateral membrane of epithelial cells in the intestines and kidneys. This results in impairment of intestinal transport and renal proximal tubule loss of the affected amino acids. The concentration of dibasic amino acids is therefore low in plasma and high in urine. Coincidentally, plasma amino acid analysis in these patients may show elevated neutral amino acids; citrulline, alanine, glycine, proline and glutamine [14, 16].

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LPI typically presents with gastrointestinal symptoms, such as vomiting and diarrhea, in addition to failure to thrive after weaning breast milk. Most patients then also develop protein aversion, leading to malnutrition, osteopenia, and anemia. Most, but not all, of the symptoms of LPI have been linked to a secondary urea cycle derangement. The dibasic amino acids arginine and ornithine are urea cycle intermediates- thus low levels of arginine and ornithine may lead to episodic postprandial hyperammonemia with resultant seizures or coma. Other neurological manifestations include hypotonia, lethargy and abnormal behavior [3, 10].

This constellation of nonspecific symptoms often leads to a delay in diagnosis [14], disease progression, and caregiver anxiety. Diagnosis requires a high index of suspicion and quantitative analysis of both plasma and urine amino acids. Plasma amino acid analysis however, may be spuriously normal if the patient is on total parenteral nutrition. Here, we describe a four-year-old patient with a delayed diagnosis of LPI.

2. Case presentation

Our patient is a 4-year-old African-American boy born full-term via vaginal delivery, following an uneventful pregnancy, with a birth weight of 2722 grams (9th percentile). He required two weeks in the Neonatal Intensive Care Unit for neonatal abstinence syndrome and poor feeding. He was only breastfed a handful of times, and various formulas were tried without success. His difficulties with feeding persisted following discharge, and soon after birth, he developed chronic diarrhea. Throughout his first year of life, he also suffered from multiple respiratory tract infections. His motor development was appropriate while both social and language development were advanced; he was very talkative and interactive. He first presented at two years of age with rectal prolapse. Given his history and presentation, cystic fibrosis was considered (frequency of 1:15,000 in the African American population) [15], but sweat chloride testing was negative. He went on to develop recurrent rectal prolapse, requiring a surgical reduction at three years old. The cause of the rectal prolapse was likely diarrhea and malnutrition, secondary to his underlying diagnosis [17].

At three years of age, he was again admitted for emesis and weight loss in the setting of chronic diarrhea and worsening abdominal distention. It was reported that despite his persistent weight loss, he typically had a good appetite. His family denied both generalized protein aversion and episodes of severe illness following protein-rich meals. At the time of presentation, he weighed 13.2 kg (3.2 percentile) and was 93 cm (1.6 percentile) tall. His hair had gradually become straighter and lighter than was typical for him over the preceding year. He had significant hepatomegaly, with a liver span of 18 cm on ultrasound. Laboratory examinations demonstrated mild anemia, hypokalemia and hypoalbuminemia in the setting of transaminitis with a normal ammonia. Radiographs of his femurs and hands bilaterally, in addition to lumbosacral spine and pelvis, all demonstrated osteopenia. An echocardiogram showed mild left atrial enlargement, a small pericardial effusion and respiratory mitral inflow variability, all consistent with constrictive pathophysiology, thought to be secondary to his systemic processes. Congenital disorders of glycosylation were considered, however carbohydrate deficient transferrin levels were only slightly elevated, inconsistent with congenital disorders of glycosylation, though possible in cases of hereditary fructose intolerance. Urine amino acids were significant for elevated lysine and ornithine, though not to the extent expected in lysinuric protein intolerance. He was discharged with a working diagnosis of hereditary fructose intolerance, instructed to follow a fructose-free diet.

Though he had initially improved on the fructose-free diet, after three days, his condition worsened. He developed food aversion and began spiking intermittent fevers. He was readmitted two weeks later with fever, decreased oral intake, weight loss, worsening abdominal distension and pain following an

Table 1
Results of plasma amino acids analyses before and after TPN

Amino acid	Level (μmol/L) before TPN	Level (μmol/L) after TPN	Reference range
Arginine	15	121	10–111
Ornithine	24	146	22–120
Lysine	65	139	42–284
Glycine	318	421	86–376
Citrulline	6	16	10–46
Glutamine	213	416	254–736

Table 2
Results of urine amino acids analyses before and after TPN

Amino acid	Level before TPN (mmol/mol of creatinine)	Level after TPN (mmol/mol of creatinine)	Reference range
Arginine	60	1027	16–67
Ornithine	147	866	0–82
Lysine	2620	8696	19–539

additional episode of rectal prolapse. The 60 most common *CFTR* mutations were tested at this time and were all negative. Plasma and urine amino acids were collected and the patient was started on empiric citrulline while awaiting results (Table 1, Table 2).

Targeted DNA Sequencing looking for *SLC7A7* gene variants was ordered, and was negative for any pathogenic mutations. However, deletions, duplications and intronic variants will not be detected by this testing. LPI can be caused by deletion or duplication of *SLC7A7* in approximately 15–20% of patients; however, a deletion/duplication assay could not be obtained [6]. Biopsies of the middle and distal esophagus, stomach, duodenum, terminal ileum and ascending and transverse colon were significant only for a focal increase in lamina propria eosinophils in the duodenum. Liver biopsy was significant for moderate to severe steatosis of the parenchyma, focal mild cholestasis, mild inflammatory infiltrate with scattered eosinophils and fibrosis. Both an upper GI series and contrast enema were completed but unrevealing.

Following protein restriction and initiation of citrulline to correct for the intracellular defects of arginine and ornithine, carnitine was also started as levels were found to be low. On this regimen, our patient's condition improved. His family reports he has had significantly more energy and his hair has begun to darken. Follow-up after 5 months showed a significant improvement regarding his nutritional status. His weight is 17.1 (45th percentile) but his height is still at 1st percentile.

3. Discussion

Here, we describe a 4-year-old African-American boy with failure to thrive, chronic diarrhea, and recurrent rectal prolapse who had a delay in diagnosis of LPI. His case raises awareness of the fact that the presentation can be confused with that of cystic fibrosis or fructose intolerance. Patients may not present with obvious protein aversion, so a high index of suspicion is necessary to make the diagnosis. Urine amino acids show elevation of lysine and ornithine, but this can be lower than in previously reported cases, particularly in the setting of malnutrition. Initiating treatment can improve clinical

course and lead to significant improvement in weight and height. Our case adds insight into the clinical presentation of LPI and provides the opportunity to review medical management and the latest research in treatment.

Several diagnostic pearls can be learned from our patient. First, the initial urine amino acid profile showed only a modest elevation of dibasic amino acids (Table 2). Close to normal concentration of urine amino acids have been occasionally described at presentation [10]. Interestingly, our patient showed a typical urine amino acid profile once his nutritional status had improved (Table 2). Second, his initial plasma amino acid profile revealed that the dibasic amino acid concentrations were within the normal range (Table 1), although in all cases, close to the lower limit of normal; interestingly, previous publications have shown a borderline significant association between lysine levels close to the lower limit of normal and a poor prognosis [10]. Third, upon institution of total parenteral nutrition, his plasma amino acid analysis showed increased concentrations of arginine and ornithine, as opposed to the expected decreased plasma concentration of those amino acids (Table 1). It has been previously shown that patients with LPI who undergo continuous infusion of arginine and ornithine show a higher plasma concentration of both amino acids when compared to control individuals undergoing the same infusion regimen, indicating decreased metabolic clearance for dibasic amino acids [18]. The continuous infusion of amino acids in the form of total parenteral nutrition thus alters the typical plasma amino acid profile expected in LPI, as the decreased metabolic clearance trumps the increased urinary excretion of dibasic amino acids.

LPI can present at any age but most often does so after weaning breast milk. It typically presents as non-specific GI symptoms such as vomiting and diarrhea, or as short stature and failure to thrive. Children with LPI usually have protein aversion and describe feeling sick after protein rich meals, leading to malnutrition and failure to thrive. Hyperammonemic crises should also raise the suspicion for LPI which can be distinguished from urea cycle disorders by increased excretion of dibasic amino acids in the urine [16]. Additionally, our patient had multiple rectal prolapses which can occur in the context of chronic diarrhea and malnutrition [17].

The protein malnutrition that occurs in LPI is thought to be the cause of various growth irregularities seen in this patient population, including failure to thrive, growth delay and short stature. Several groups have studied the impact of either amino acid supplementation or exogenous hormone administration in these children, with interesting results. Awrich and colleagues [1] found that the velocity of both height and weight gain improved with amino acid supplementation. They found that supplemental citrulline, together with lysine, had robust effects on growth, as did the combination of arginine and lysine, though the latter was to a lesser extent. The role of arginine supplementation alone in LPI patients has also been investigated and was associated with improvement in growth [7].

More recent studies have considered the efficacy of growth hormone administration in this population. Esposito et al. [4] reported on a patient with comorbid LPI and growth hormone deficiency, who experienced a significant improvement in growth velocity following exogenous hormone administration. They suggested a possible link between growth hormone deficiency and LPI, causing them to occur concomitantly, as persistently low arginine and lysine levels may hinder growth hormone secretion. Niinikoski et al. [13] later investigated the benefit of exogenous growth hormone in LPI patients without growth hormone deficiency and found similar results. Supplementation promoted growth, though bone age did not catch up to chronological age. Adult height had not been reached by the end of either study, and thus could not be reported on, but these results were promising as improved growth had been seen without adverse effects from long-term exogenous hormone administration.

Interestingly, however, Evelina et al. [5] found that in their patient with LPI, growth hormone administration did not lead to an improvement in growth. Despite supplementation, IGF-1 levels remained low, possibly indicating a defect in the coupling of growth hormone to IGF-1 production. This final study highlights the fact that despite previous successes, additional research into both amino

acid and growth hormone supplementation is necessary. It will be important to further elucidate the activity of both growth hormone and IGF-1 in LPI patients.

There are many long term complications associated with LPI which need to be monitored and managed in an effort to improve quality of life. Pulmonary involvement, in particular, can lead to major complications, significantly impacting prognosis. This includes early and persistent pulmonary alveolar proteinosis, which can be diagnosed via bronchoalveolar lavage or lung biopsy, pulmonary fibrosis and respiratory insufficiency which, at times, can be fatal. Although the role of lysine and cationic amino acids in pulmonary alveolar proteinosis has not been clearly defined, there is some evidence that patients with higher plasma lysine levels may have poorer prognoses [10]. A recent study demonstrated the role of $y+$LAT1 protein in inflammation control. Upon SLC7A7 silencing there was an increase in production of cytokines by macrophages and epithelial cells which could be the reason for pulmonary complication [24]. Renal involvement may occur and starts as proximal tubulopathy with proteinuria and hematuria, which can later progress to chronic renal failure. Renal function surveillance should be emphasized [14]. As well, hemophagocytic lymphohistiocytosis, characterized by hepatosplenomegaly, fever and cytopenia, can develop secondary to LPI, causing excessive activation of the immune system and secretion of pro-inflammatory cytokines [20].

The main goals of LPI management are to provide adequate nutrition to the patient while also preventing hyperammonemia. It is recommended that dietary protein be restricted to 0.7–1.2 g/kg/day and supplemental citrulline be given, limited to 100 mg/kg/day [14]. Ammonia scavengers can also be used if needed, based on plasma ammonia and glutamine levels. Carnitine supplementation is also required as individuals with LPI tend to be carnitine deficient [21]. Due to the low plasma lysine levels in this population, lysine supplementation can also be given with L-lysine hydrochloride, dosed at 0.05–0.55 mmol/kg [9], although there is limited lysine absorption given the transport defect. Hyperammonemic crises are managed similar to those of urea cycle disorders, and include protein intake cessation, initiation of intravenous fluids and ammonia scavengers, citrulline supplementation, and plasma and urine collection for diagnostic purposes [8].

In summary, we present useful clinical diagnostic pearls for the diagnosis of LPI. Urine amino acids can be normal in this condition in the setting of malnutrition, a common complication of the disease. Additionally, it has been previously shown that the plasma arginine and ornithine concentration is higher in LPI subjects than in controls after amino acid infusion, given decreased metabolic clearance. In the setting of enteral nutrition, the plasma concentration of dibasic amino acids is decreased given impaired intestinal absorption; in the setting of TPN, however, the decreased metabolic clearance overcomes the increased urinary excretion, leading to dibasic aminoacidemia. Due to the rarity of this disease, further case reports and studies will help clinicians recognize and optimally treat LPI.

Conflicts of interest

The authors have no conflicts of interest to disclose.

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