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Hyperammonemia with Citrullinemia

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Two cases of hyperammonemia with elevated citrulline are reported, one resulting from a deficiency of pyruvate carboxylase and the other from a partial deficiency of argininosuccinate synthetase. Diagnosis was based on clinical, biochemical and amino acid profiles. The utility of amino acid determinations in hyperammonemia suspected to underlie an inborn error of metabolism is emphasized.

Key words: *Citrulline, Hyperammonemia.*

Inborn errors of metabolism (IEM) are of concern in India, the spectrum being wide, varied and poorly diagnosed. Population based studies indicate tyrosinemia, maple syrup urine disease and phenylketonuria to be the commonest inborn errors of amino acid metabolism among newborns in India(1). Amino acid disorders seen in a tertiary care hospital would be more varied, as they would include sick children. We report 2 cases of citrullinemia with hyperammonemia which represent the disruption of different metabolic pathways.

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Case reports

Patient 1. A neonate presented at 20 hours of age with poor feeding and respiratory distress. He was born to consanguineous parents and two previous siblings had died in the neonatal period with similar complaints. There was no history of birth asphyxia or any risk factors for sepsis. The blood counts, blood sugar, electrolytes and chest *X*-ray were normal and blood culture was sterile. On examination he was in severe respiratory distress with weak peripheral pulses and a capillary refill time >4 seconds. Arterial blood gas revealed severe metabolic acidosis with an anion gap of 35. Serum urea was 37 mg%, plasma ammonia 294 µg/dL (normal 20-80 µg/dL) and serum lactate 4.5 mM (normal 0.3-1.3mM). Urine was negative for reducing substances and ketones. Amino acid profiles determined by high performance liquid chromatography, revealed raised levels of citrulline (440 moles/L), proline (410 moles/L) and lysine (680

moles/L). He was resuscitated with normal saline, provided ionotropic support and metabolic acidosis corrected with intravenous sodium bicarbonate. He was also started on therapeutic doses of vitamin B_{12}, pyridoxine, riboflavin and thiamine. The neonate improved and was started on nasogastric feeds on the third hospital day. He deteriorated 4 days later with the same manifestations and died on the 8th day of life.

Patient 2. A 15-year-old boy presented with a history of episodic vomiting associated with subacute encephalopathy from the age of one year. There were 2-3 months of asymptomatic periods between the episodes. He was born of non-consanguineous parents at full term and had a normal perinatal period with no evidence of birth asphyxia. His developmental history was normal. During hospital stay he had 2 episodes of convulsive seizures. In view of his clinical history he was investigated for an IEM. Investigations revealed high blood ammonia (288 µg/dL), markedly elevated levels of citrulline (2200 µmoles/l, normal 1-55 µmoles/L), normal ornithine (35 µmoles/L) and low blood urea (12 mg/dL). The urinary orotic acid was elevated (26 mg/g creatinine, normal $\leq$ 20 mg/g creatinine). He was treated with anti-convulsants and sodium benzoate (250 mg / kg body weight /day), advised a restricted protein diet and continues to do well a year later.

Discussion

Hyperammonemia accompanied by citrullinemia can occur either from a disruption of energy metabolism due to an absence of pyruvate carboxylase or from a defect of the urea cycle.

Pyruvate carboxylase deficiency (EC 6.4.1.1.) (McKusick 266150) is characterized by lactic acidemia from a lack of gluconeogenesis and the Cori Cycle as well as by low aspartate and the accumulation of the urea cycle intermediate, citrulline. This elevated level of citrulline results in an increase of the other urea cycle intermediates arginine and ornithine. The deficiency also features elevated levels of blood proline, alanine and lysine, from an increased availability of pyruvate. Elevated alanine also reflects the associated hyperammonemia. Although pyruvate and orotic acid levels were not determined and moderately elevated citrulline may suggest a argininosuccinate lyase deficiency in Patient 1, the metabolic profile especially the severe metabolic acidosis and lactic academia do not support a urea cycle disorder(2) but more likely a deficiency of pyruvate carboxylase in this patient. Pyruvate carboxylase deficiency presents in 3 clinical forms, infantile, severe neonatal and benign. A partial deficiency of pyruvate carboxylase that is ultimately fatal underlies the infantile form (Type A/N American form) while enzyme activity is completely absent in the severe neonatal (Type B) form (French (or UK) phenotype) (3-5). Low levels of the enzyme (upto 5%) with a benign clinical course and no major metabolic upsets characterize the benign form of the enzyme deficiency(6,7). The clinical and biochemical profile and death of the neonate would suggest Type B pyruvate deficiency in Patient 1.

Hyperammonemia with critically elevated levels of citrulline indicates a deficiency of argininosuccinate synthetase (EC 6.3.4.5) in the urea cycle, the classical citrullinemia type II (CTLN2; McKusick 603471). Citrullinemia from a urea cycle defect will also feature low to normal levels of the other urea cycle intermediates and an increased excretion of orotic acid from an increased availability of carbamoyl phosphate. The hyperammonemia that accompanies a urea cycle defect will

reflect in high blood glutamine and alanine. The notable citrullinemia and biochemical profile of Patient 2 suggests a deficiency of argininosuccinate synthetase.

Although enzymatic determinations and DNA analysis are considered confirmatory for metabolic and genetic disorders, these two cases of citrullinemia were delineated from their clinical, biochemical and different amino acid profiles, into an energy disorder of a pyruvate carboxylase deficiency and a urea cycle defect of partial argininosuccinate synthetase deficiency. The severe citrullinemia of argininosuccinate synthetase deficiency is in fact considered sufficient confirmation for the disorder(8). In a partial deficiency of argininosuccinate synthetase the disease presents when the system is challenged with a protein load. Until such events, metabolism is normal as evidenced by the episodic history of Patient 2, explaining the normal development of this patient.

Pyruvic acidemia has been reported in a child from India by Tibrewala, *et al.*(9) based on characteristic clinical and non-enzymatic biochemical profiles. There is one report in an Indian neonate of a urea cycle defect of argininosuccinate synthetase deficiency by Balsekar, *et al.*(10) also based on clinical and biochemical profiles. Arguably citrullinemia I, II, III, must be more common in India than documented, the inability to investigate limiting detection. In the context we recommend amino acid determinations in cases of hyperammonemia suspected to underlie an IEM.

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