Significance of Newborn Screening for Citrullinemia

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Introduction

Citrullinemia is a rare, genetically-inherited condition that belongs to the group of metabolic disorders called urea cycle disorders. Since inheritance of this condition is autosomal recessive, neonates of both sexes could be equally affected. There are 6 different urea cycle disorders based on the enzymes that are deficient in the urea cycle pathway. Citrulline is the product of condensation of carbamoyl phosphate and ornithine, which is catalyzed by ornithine transcarbamylase enzyme. Citrulline, under normal circumstances, is condensed with aspartic acid to form argininosuccinic acid. This reaction is mediated by the enzyme argininosuccinic acid synthetase I. Deficiency of this enzyme causes urea cycle disorder leading to accumulation of ammonia in blood stream, which in turn causes central nervous system disorders.

Incidence of citrullinemia or any metabolic disorder, in India, is not known due to lack of universal screening for metabolic disorders in neonates. We discuss 3 cases with neonatal citrullinemia who presented in our hospital during 2012 to 2015.

Case Reports

Case 1

A male child was born to a nonconsanguineous couple 7 years ago, who currently is alive and healthy. Their second child, a male neonate weighing 3.2 kg, was born vaginally with APGAR scores of 9 and 10 at 1 and 5 minutes, respectively. On day 3 of life, the neonate developed lethargy and tachypnea and was admitted to neonatal intensive care unit (NICU) in a local teaching hospital. Over the next few days, the neonate deterio-

rated with multiorgan failure and died. According to the discharge diagnosis, it was thought to be neonatal encephalopathy of unknown cause or probably of perinatal origin. Lately, their third child, a female neonate weighing 3.6 kg, was delivered at our hospital. The neonate was closely monitored. As per our protocol, the neonate's blood was collected 36 hours after birth for metabolic screening with continuous monitoring and the mother continued to breastfeed. Sixty hours after birth, the neonate developed tachypnea and convulsions; meanwhile, we received the screening results revealing citrullinemia type 1 due to deficiency of an enzyme argininosuccinate synthetase (ASS). Laboratory reports revealed hyperammonemia (636 umol/L) and high lactate level suggestive of urea cycle defect. Tandem mass spectrometry (TMS) detected abnormal elevation of citrulline with deficiency of argininosuccinic acid synthetase and confirmed citrullinemia type 1. Despite instituting all the corrective measures, including treatment for hyperammonemia along with stopping breastfeeding, the neonate progressed rapidly to death.

Neonatal screening disclosed the cause of death of the third child to be citrullinemia. The parents realized their second child had similar conditions and felt they had a "closure" to their enigma.

Case 2

A female neonate was born vaginally at 39 weeks of gestation to a primigravid woman (nonconsanguineous marriage). The antenatal period was uneventful. The neonate was floppy limp at birth, requiring positive pressure ventilation for 2 minutes. The neonate required oxygen to maintain saturations > 95% at 10 minutes of life. Initially, the neonate was managed in NICU keeping a possibility of transient tachypnea of the newborn; later, the neonate was shifted to postnatal ward on day 2 of life, after respiratory distress had settled and sepsis workup was found to be negative. The neonate was accepting breastfeeds well and found to be euglycemic. The neonate looked pale with poor perfusion on day 3 of life and was thus transferred back to NICU and was ventilated; it also required inotropic support. Echocardiogram showed decreased ejection fraction and poor myocardial contractility. Blood gas showed metabolic acidosis, which persisted. The neonate showed generalized tonic–clonic seizures on day 4 of life, which responded to sodium valproate and there was evidence of cerebral edema on cerebral ultrasound scan. Plasma ammonia concentration was found to be 1000 µmol/L. Plasma quantitative amino acid analysis showed absence of argininosuccinic acid and concentration of citrulline > 1000 µmol/L (normal range, < 50 µmol/L). TMS done was confirmatory for citrullinemia type 1. The neonate was treated with sodium benzoate and arginine. It initially responded to treatment but due to deteriorating clinical condition, had to be hemodialysed. The neonate demised on day 7 of life despite best efforts. Blood was collected for DNA storage. Genetic evaluation and microarray analysis revealed mutation in *ASS1* gene on chromosome 9.

Case 3

A nonconsanguineous couple had lost their first baby, born through cesarean section, on day 3 after birth. The cause of death was thought to be sepsis. Later, the second child, a female neonate, was delivered in our hospital at 38 weeks + 4 days of gestation. The neonate was stable and on breastfeeds. At 25 hours after birth, due to the suspicion of inborn error of metabolism considering the previous neonatal demise, a dried blood sample on filter paper was sent for analysis and the breastfeeds were stopped. The preliminary result pointed toward the presence of citrullinemia. The neonate was transferred to NICU for further management. Serum ammonia was 216.7 µmol/L, plasma lactate was 59.8 mg/dL at 25 hours after birth. Urine analysis for amino acids and organic acids were negative. Liver function tests were within normal limits. Administration of oral L-arginine and sodium benzoate were commenced after which the serum ammonia levels decreased. Confirmatory results of citrullinemia type 1 were available at 48 hours of life. Hence, strict dietary plan was initiated. Initially, the neonate did not tolerate this formula and vomited a few times. Serum ammonia rose to a maximum of 360.7 µmol/L on day 8 of life. On day 10, a protein-free, carbohydrate-based

formula (Pro-Phree, Abbott) was initiated. However, the neonate was continued on the special formula. The neonate underwent liver transplantation at 10 months of age; the maternal grandmother was a complete match for the live liver donor. Currently, she is 24-month healthy infant and doing very well developmentally and growing up like any normal child.

Discussion and Conclusion

Newborn screening is a public health program, which is in its infancy in India. Though it is nearly 50 years since its inception in many parts of the world, it will be few more years before India will have the data and resolve to justify public health program of screening for metabolic disorders. Inborn errors of metabolism (IEM) remains an enigma for many pediatricians, but few realize that they encompass a varied group of disorders ranging from amino acidurias to fatty acid oxidation defects and organic acidurias. There is enough anecdotal evidence and some academic publications to suggest that India has reasonably high incidence of urea cycle defects accounting for many neonatal deaths.[1,2]

The 3 cases discussed here are presented for several reasons:

1. Citrullinemia is a relatively rare disorder and the number of neonates who have survived with this neonatal manifestation are a handful in all countries, across the world, since most neonates die even before the diagnosis is made or suspected.
2. As per the guidelines from the Centers for Disease Control and Prevention, newborn screening for metabolic disorders has to be performed anytime between 36 to 72 hours after birth. For many neonates, this time lag is enough for the breast milk with its protein content to cause hyperammonemia and death, as seen in case 1.
3. If there is a case history of a sibling's death of unknown cause within the first week of life, a pediatrician should suspect metabolic disorder. In many such instances, it is erroneously classified as sepsis. However, urea cycle defect could be a possible cause and leads to hyperammonemia and death.
4. If any serious suspicion of this group of metabolic disorder is considered, the treating pediatrician should collect blood sample of the neonate after 24 hours of good breastfeeding for analysis of serum ammonia and lactate levels and metabolic screening by TMS. If the neonate has hyperammonemia, the feeds should be stopped and intravenous glucose should be initiated immediately; in addition, treatment for hyperammonemia should be started while awaiting results of metabolic screening, which generally may take anywhere between 24 to 96 hours.
5. Molecular genetics diagnostic tests are confirmatory tests for this condition. This can be followed by counseling of the parents for subsequent pregnancy. The results of genetic evaluation found in case 2—sequence analysis of *ASS1* gene on chromosome 9—is in accordance with previous data.[3] There have been case reports of genetic diagnosis in parents with neonate having died due to citrullinemia.[4] Thus, parents should be counseled to undergo genetic screening for citrullinemia before they plan subsequent pregnancy.

The early diagnosis of citrullinemia in prenates or neonates will not only give a closure and meaningful answer to the parents, but also help them to plan for future pregnancies. In addition, it gives a sense of satisfaction for the treating pediatrician.

References

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