EVALUATION OF PYRIMIDINE PATHWAY METABOLITES AS NOVEL BIOMARKERS FOR ORNITHINE TRANSCARBAMYLASE DEFICIENCY

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Background: Orotic acid has been used as a potential marker in newborn screening (NBS) for ornithine transcarbamylase deficiency (OTCD), but its clinical utility remains uncertain, as several cases—including both early and late-onset forms—have shown normal orotic acid levels in dried blood spots (DBS). One possible explanation is the low sensitivity of orotic acid in mass spectrometry, leading to reduced accuracy and possible misidentification due to isobaric compounds in flow injection-MS/MS (FI-MS/MS). In proximal urea cycle disorders, excessive mitochondrial carbamoyl phosphate may leak into the cytosol and enter the pyrimidine biosynthesis pathway. There, it is converted to carbamoylaspartic acid and subsequently to dihydroorotic acid by the CAD enzyme, and finally converted to orotic acid by dihydroorotate dehydrogenase. We applied liquid chromatography–tandem mass spectrometry (LC-MS/MS) to accurately quantify orotic acid and pyrimidine metabolites, in order to explore a novel biomarker for OTCD.

Methods: We performed hydrazine-based derivatization and measured pyrimidine metabolites (carbamoylaspartic acid, dihydroorotic acid, orotic acid) and N-Acetylglutamic Acid in DBS using LC-MS/MS. DBS samples were obtained from the following individuals: healthy controls (n = 2), a patient with late-onset OTCD (n = 1), a patient with neonatal-onset OTCD during hyperammonemia (n = 1), a patient with hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome during hyperammonemia (n = 1), and a patient with neonatal-onset carbamoyl phosphate synthetase 1 deficiency (CPS1D) (n = 1).

Results: Elevated orotic acid levels were observed only in the patients with neonatal-onset OTCD during hyperammonemia (1.38 μM) and HHH syndrome during hyperammonemia (2.61 μM). The other individuals, including controls, the CPS1D and late-onset OTCD cases, showed undetectable orotic acid levels. Dihydroorotic acid and carbamoyl aspartate, similar to orotic acid, were elevated during hyperammonemia in patients with neonatal-onset OTCD and HHH syndrome (0.216 μM and 1.37 μM). In contrast, these metabolites remained low in controls, late-onset OTCD and CPS1D, with levels ranging from 0.001 to 0.031 μM for dihydroorotic acid and from 0.17 to 0.23 μM for carbamoyl aspartate. However, a limitation

of this study is the lack of samples from patients with N-acetylglutamate synthase deficiency (NAGSD) and CPS1D during hyperammonemia.

Conclusions: This pilot study demonstrates that LC-MS/MS with hydrazine derivatization allows quantification of pyrimidine metabolites in DBS. Further evaluation in larger cohorts is warranted to clarify their diagnostic potential in newborn screening for proximal urea cycle disorders.