Hyperammonemia: Diagnostic Experience At The Metabolism Laboratory

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Citation

Abstract

Context: Ammonia is produced normally from the catabolism of amino acids through the urea cycle occurring in the hepatocytes. At concentrations > 60 uM/L, clinical signs of Hyperammonemia are seen in children, which are manifested as lethargy, vomiting, and can progress to coma & death. Common causes of Hyperammonemia include Urea cycle defects, Organic acidemias or hepatic dysfunction.

Aim: To evaluate amino acid and organic acid profiles of Hyperammonemia (> 80uM/L) cases using High Performance Liquid Chromatography (HPLC) & to diagnose the underlying metabolic disorder, if any.
Setting: All cases of Hyperammonemia referred to Metabolic Disorders Laboratory, Amrita Institute of Medical Sciences, Kochi from May 2003 were considered.

Materials: HPLC was used for quantitation of amino acids and organic acids. Amino acids were quantitated using Phenylisothiocyanate method & organic acids on C18 column with UV detection.

Results & Statistics: Of a total of 171 referrals, 78 cases were neonatal presentations. 42 were infants and 51 cases were > 1 year of age. 76% of the cases (130 cases) were diagnosed to have some sort of metabolic disorder. 29% cases were due to Urea cycle defects. While 27% & 24% respectively were contributed by Propionic acidemia & Methylmalonic acidemia, 9% were due to Pyruvate metabolism defects. Multiple carboxylase defects, Branched Organic acid defects, Lysinuric protein intolerance & Hypervalinemia together made up 9%. 41 cases which were inconclusive even after amino acid and organic acid HPLC could probably have been diagnosed with mutation analysis.

Conclusion: The data shows that these disorders are not uncommon, but are presented as encephalitis, cerebral palsy, epilepsy etc. Clinicians should thus be aware of the various manifestations of these disorders. Estimation of ammonia levels is the first step towards diagnosis.

INTRODUCTION

Ammonia, normally produced from catabolism of amino acids, is a potentially lethal neurotoxin. The conversion of ammonium to urea, which involves urea cycle, occurs in the mitochondrial and cytosolic compartment of hepatocytes. Urea, which contains two nitrogen atoms, is synthesized from aspartate and ammonium mobilized from various sources as detailed above. The urea cycle, also called Krebs-Henseleit cycle, typically involves six enzymatic reactions. The first 3 steps occur in the mitochondria and the next 3 steps occur in the cytoplasm. [1, 2]

The concentration of free ammonia in blood is very tightly regulated and is exceeded by two orders of magnitude by its physiologic derivative, urea. The normal capacity for urea production far exceeds the rate of free ammonia production by protein catabolism under normal circumstances, such that any increase in free blood ammonia concentration is a reflection of impairment of urea cycle function or fairly extensive hepatic damage. [1] Clinical signs of hyperammonemia occur at concentrations > 60 micro mol/L and include anorexia, irritability, lethargy, vomiting, somnolence, disorientation, asterixis, cerebral edema, coma, and death; appearance of these findings is generally proportional to free ammonia concentration, is progressive, and is independent of the primary etiology. Common causes

of hyperammonemia include genetic defects in the urea cycle, organic acidemias ("secondary urea cycle dysfunction"), as well as genetic or acquired disorders resulting in significant hepatic dysfunction. [2] Hyperammonemia occurs commonly due to defective detoxification in the liver due to a various inborn errors of metabolism and rarely due to excess production in kidneys and intestine. [6] Acute and chronic liver disease resulting in hyperammonemia are also known. Table-1 lists some of the well-recognized causes of hyperammonemia. [2, 3]

Figure 1

Table 1: Causes of hyperammonemia

Deficiencies of Urea cycle enzymes
Carbamyl phosphate synthetase (CPS)
Ornithine transcarbamylase (OTC)
Argininosuccinic acid synthetase. (AS)
Argininosuccinase. (AL)
Arginase
N-acetyl glutamate synthetase (NAGS)

Organic Acidemias
Propionic acidemia
Methylmalonic acidemia
Isovaleric acidemia
Ketothiolase deficiency
Multiple carboxylase deficiency
Glutaric acidemia-type-II
3-hydroxy, 3-methylglutaric acidemia
Other rare organic acidemias

Others Inborn errors
Lysinuric protein intolerance
Hyperammonemia-Hyperornithinemia- homocitrullinemia syndrome (HHH)
Periodic hyperlysinuria with hyperammonemia
HYpervalinemia
Transient hyperammonemia of newborn

Miscellaneous causes
Acute or Chronic liver disease due to any cause
Drugs: Sodium valproate
Urinary tract infections due to Urease producing organisms

Enzyme deficiencies of all the five classical urea cycle enzymes result in persistent or episodic hyperammonemia along with accumulation of various intermediates of urea cycle depending on the site of metabolic block. N-acetyl glutamate synthetase deficiency also leads to ammonia accumulation, as N-acetyl glutamate is an allosteric activator of CPS enzyme. [4] The other metabolic disorders affect urea cycle in novel ways. In organic acidemias, the accumulated toxic organic acids and their esters in the mitochondria inhibit CPS-I. Inhibition of N-acetyl glutamate synthetase by propionic acid is another proposed mechanism for hyperammonemia in propionic acidemia. [5] In lysinuric protein

intolerance, defective transport of dibasic amino acids leads to low serum ornithine level (in addition to low lysine and

arginine), which is insufficient to support activity of OTC. [6] In hyperammonemia-hyperornithinemia-homocitrullinuria syndrome, intra-mitochondrial ornithine deficiency impairs the activity of OTC. [7] Failure of immediate closure of ductus venosus after birth is thought to result in transient hyperammonemia of newborn as portal blood bypasses the liver. [8] Sodium valproate increases ammonia levels by inhibition of CPS-I enzyme and by activating renal glutaminase activity promoting ammonia formation in kidneys. [9] Addition of topiramate to valproate may increase the propensity to develop hyperammonemia by inhibiting glutamate synthetase in brain. [10] Finally, symptomatic hyperammonemia can also occur in urinary tract infections due to urease producing organisms such as proteus, resynthesising ammonia from urea. [11]

METHODS

We have used High Performance Liquid Chromatography (HPLC, Shimadzu LC 10 AT VP)) with a Diode array detector (SPD M10 AVP) for quantitations of aminoacids & organic acids. Aminoacids were quantitated using the Phenylisothiocyanate method (PICOTAG) from Waters Corporation. Organic acids were quantitated on the HPLC using a C-18 (150x4.6mm, particle size 5μm) column; mobile phase of 99:1 potassium phosphate buffer (pH 2.5, 50mM): acetonitrile. Flow rate was set of 1.5 ml/minute and detection was at 210nm. All cases of hyperammonemia (> 80 micromole/liter) referred to the Metabolic Disorders Laboratory from May 2003 were evaluated for their aminoacid and organic acid profiles. The distribution of the patients age-wise is shown in Table 2.

Figure 2

Table 2: Age wise distribution of referrals

TYPE	NUMBERS
NEONATAL PRESENTATIONS <28days	78
BETWEEN 1month -1year	42
FROM 1year-5 years	36
FROM 5 years OR ABOVE	15

RESULTS

Results by summation of all cases are presented in Table-3.

Figure 3

Table 3: Metabolic Disorders producing hyperammonemia evaluated at Metabolic Disorders Laboratory

DISORDER	NUMBER OF CASES	TECHNIQUE EMPLOYED FOR DIAGNOSIS
Urea Cycle Disorder	38	HPLC for amino acids, enzyme assay for OTC, Arginase
Pyruvate Metabolism Defect	12	HPLC for amino acids, organic acids
Propionic Acidemia	36	HPLC for organic acids
Methylmalonic Acidemia	32	HPLC for organic acids
Multiple Carboxylase Deficiency	6	HPLC for amino acids, organic acids
Lysinuric Protein Intolerance	1	HPLC for amino acids
Branched Chain Organic Acid Defects	4	HPLC for amino acids, organic acids
Hypervalinemia	1	HPLC for amino acids
Inconclusive	41	HPLC for amino acids, organic acids

DISCUSSION

Disorders causing hyperammonemia constitute an important group of potentially treatable metabolic disorders manifesting predominantly with neurological manifestations in early life. Thus, because of the neurotoxic implications of hyperammonemia and the typical absence of other specific laboratory abnormalities, appearance of clinical signs compatible with hyperammonemia should initiate an emergent search for elevated blood ammonia concentration. A value of more than 150 mµol/L (260 µg/dl) in infants and more than 100 µmol/L (175 µg/dl) in older children and adults warrants further biochemical investigations. [3] Though the plasma concentration of ammonia in a symptomatic child is usually 3 or more fold elevated, lower levels can produce symptoms and rarely, raised glutamine alone without hyperammonemia can occur as the biochemical marker of urea cycle dysfunction. After confirming hyperammonemia, the first step is to look at serum bicarbonate, sodium, chloride and urine ketones. [2, 3, 12] Acidosis usually indicates the possibility of organic acidemias whereas urea cycle disorders often present with respiratory alkalosis. If acidosis is absent (respiratory alkalosis is often present), the second step is to look at the concentrations of amino acids in blood and urine. [2, 3,12] Diagnostic criteria & cutoff values for aminoacid concentrations in plasma are well established [2]. If the amino acid profile is non-diagnostic, the third step is to look for urine orotic acid. This can be done as part of organic acid analysis or as specific assay for orotic acid. [2, 3, 12] Orotic aciduria is found in patients with OTC deficiency. In a patient without a diagnostic amino acid profile and without orotic aciduria, the usual diagnosis is CPS deficiency. NAGS deficiency closely resembles CPS deficiency, but is very rare. Transient hyperammonemia of the newborn also has similar features but is much rare in the last two decades for unknown reasons. Citrulline levels are absent or present in trace in CPS and NAGS deficiencies whereas in transient hyperammonemia of newborn, it is mildly elevated. Low citrulline levels can be viewed as a marker for mitochondrial urea cycle enzyme deficiencies, namely OTC, CPS and NAGS. [3, 13]

Despite all these investigations, the diagnosis may remain inconclusive especially in patients with episodic symptoms. A re-evaluation during a crisis may possibly yield a diagnosis. We presume the forty-one inconclusive cases are in this category. A genomic / mutation analysis could have probably yielded the diagnosis.

As evident from the data showed, these disorders are not uncommon and they masquerade as common disorders like encephalitis, cerebral palsy and epilepsy. Clinicians should be aware of the various manifestations of these disorders. High index of suspicion and estimation of ammonia levels in appropriate clinical setting is the first step towards the diagnosis. A comprehensive biochemical evaluation should follow to arrive at the final diagnosis.

Though a variety of therapeutic options are available, the developmental outcome is not optimal in majority of cases. Late onset cases with episodic symptoms have better prognosis. Early diagnosis and strict regulation of nitrogen metabolism is crucial in improving the outcome.

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