Molecular Diagnosis of Urea Cycle Disorders: Current Global Scenario

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Urea cycle disorders are a group of inborn error of metabolism, characterized by hyperammonemia, metabolic alkalosis and clinical features of encephalopathy. These are among the commonest types of inborn errors of metabolism with a frequency of 1 in 8,000 to 1 in 30,000 in different population. This encompasses 5 major disorders, corresponding with deficiency of each step in the urea cycle, namely ornithine transcarbamoylase (OTC) deficiency, argininosuccinate lyase (ASL) deficiency, carbamoyl phosphate synthetase (CPS) deficiency, citrullinemia and argininemia. The most important clinical presentation is neurological abnormalities. The severity of UCD is correlated to extent of hyperammonemia. Early diagnosis and treatment are essential for successful patient outcome. Various modalities of treatment have been recommended; namely, treatment aimed at reducing ammonia level, including drugs like sodium benzoate and sodium phenyl butyrate, neuroprotective strategies, low protein diet, liver transplantation and hepatocyte transplantation. Molecular diagnosis is important to identify the pathogenesis of these disorders as well as it helps in prognosis. This review intends to summarize the important aspects of molecular diagnostic studies on urea cycle disorders.

Keywords: Arginase deficiency, Citrullinemia, Carbamoyl phosphate synthetase I deficiency, Hyperammonemia, Molecular diagnosis, Ornithine trans carbamoylase deficiency, Urea cycle disorders

Introduction

Urea cycle disorders (UCD) are a group of inborn errors of metabolism characterized by increase in plasma ammonia concentration above 150 µmol/L, normal anion gap and blood glucose and in most cases, metabolic alkalosis. They are among the most common types of inborn errors of metabolism. There are five main disorders, namely ornithine transcarbamoylase (OTC) deficiency, argininosuccinate lyase (ASL) deficiency, carbamoyl phosphate synthetase (CPS) deficiency, citrullinemia and argininemia. The over-all incidence of UCD is reported as 1 in 8,000 to 1 in 30,000 in different populations.

Deficiency of any of the urea cycle enzymes may result in hyperammonemia. When the block is in one of the earlier steps, the condition is more severe, since ammonia itself accumulates (“proximal UCD”). Deficiencies of later enzymes (argininosuccinate synthetase, argininosuccinate lyase and arginase) results in the accumulation of other intermediates which are less toxic and hence symptoms are less (“distal UCD”). Clinical symptoms include vomiting, irritability, lethargy and severe mental retardation. Infants appear normal at birth, but within days progressive lethargy sets in.

Important characteristics of UCD are summarized in Table 1.

Clinical features

Children are usually born full-term with no obstetric risk factors and then exhibit progressive lethargy, hypothermia and apnea related to high ammonium levels. Encephalopathy is seen and is characterized by brain edema and swollen astrocytes due to accumulation of glutamine, resulting in osmotic shifts of water into the cells. They may also present in later infancy, childhood or adulthood with episodic mental changes including lethargy and behavioral changes. Neurologic abnormalities like cerebral edema, seizures, cognitive impairment,
psychiatric illness, intellectual and developmental abnormalities are also seen. Laboratory diagnosis

Amino acid analysis and urine orotate measurement can help to distinguish between the different types of UCD. Molecular genetic analysis or enzyme assays is generally used for confirmatory diagnosis. Defects in enzymes of urea cycle are detected in neonatal blood by tandem mass spectrometry. The flow chart for diagnosing UCD is given in Fig. 1A and B.

**Hyperammonemia**

Hyperammonemia in UCDs affects central nervous system, leading to changes in mental status, brain edema, seizures, coma and potentially death. It can lead to changes in neurotransmitter levels. For example, acute hyperammonemia can lead to activation of NMDA receptors, leading to excitotoxic cell death, changes in energy metabolism and alterations in astrocyte protein expression. Elevation of glutamine and reduction of myoinositol are also observed. Chronic hyperammonemia produces adaptive responses in NMDA receptor and impairment of glutamine/nitric oxide/cGMP pathway producing alterations in cognitive functions and learning. Brain tissue is more susceptible to harmful effects of ammonium in childhood than during adulthood. Neuroprotective strategies like NMDA receptor antagonists, nitric oxide inhibitors, creatine, acetyl-L-carnitine, ciliary neurotrophic factor (CNTF) and

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme deficit</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperammonemia type I (CPSD)</td>
<td>CPS – 1</td>
<td>Very high ammonia levels in blood. Autosomal recessive. Mental retardation. Comparatively rare.</td>
</tr>
<tr>
<td>Hyperammonemia type II (OTCD)</td>
<td>OTC</td>
<td>Ammonia level high in blood. Increased glutamine in blood, CSF and urine. Orotic aciduria due to channeling of carbamoyl phosphate into pyrimidine synthesis. X-linked. Commonest type.</td>
</tr>
<tr>
<td>Citrullinemia (ASD)</td>
<td>ASS</td>
<td>Autosomal recessive. High blood levels of ammonia and citrulline (&gt;1000–5000 µM, normal 10-20 µM). Citrullinuria (1-2 g/day).</td>
</tr>
<tr>
<td>Argininosuccinic aciduria (ALD)</td>
<td>ASL</td>
<td>Argininosuccinate in blood and urine. Friable brittle tufted hair (Trichorrhexis nodosa).</td>
</tr>
<tr>
<td>Hyperargininemia</td>
<td>Arginase</td>
<td>Mildest variety. Hyperammonemia is less severe. Arginine increased in blood and CSF. Instead of arginine, cysteine and lysine are lost in urine.</td>
</tr>
</tbody>
</table>

**Table 1—Important characteristics of UCD**

Fig. 1—The flow chart for diagnosing UCD
inhibitors of MAPKs and glutamine synthetase have been used for the treatment of UCD.\textsuperscript{4,9}

**Treatment**

It is recommended that plasma ammonia level should be measured in all patients with encephalopathy. Ammonia levels >200 µmol/L increases morbidity and mortality significantly in patients with UCD.\textsuperscript{11}

Low protein diet is the most important step in the dietary management of UCD.\textsuperscript{12} Sodium phenyl butyrate and glycerol phenyl butyrate are used in the treatment of UCD.\textsuperscript{13} Sodium phenyl butyrate is a histone deacetylase inhibitor (HDACI) and is found to reduce levels of ammonia in patients with UCD. The drug has additional effects on DNA methylation, histone deacetylation and protein isoprenylation. It creates alternative pathways for nitrogen excretion. The primary metabolite, phenylacetate conjugates with glutamine in the liver and kidney to form phenylacetyl glutamine and is excreted in the urine.\textsuperscript{14}

However, the drug has found to decrease the levels of branched chain amino acids (BCAA) and it has been suggested that BCAA supplementation should be given to patients with UCD treated with sodium phenyl butyrate.\textsuperscript{14}

Sodium benzoate is another important treatment option. It is found that sodium benzoate can eliminate nitrogen in patients with UCD.\textsuperscript{15} Prenatal sodium benzoate is also postulated to have beneficial effect to the fetus.\textsuperscript{15} Treatment with benzoate and phenylacetate is found to improve survival in patients with UCD.\textsuperscript{16}

Liver transplantation is another mode of therapy.\textsuperscript{17,18} It has been found to reduce hyperammonemia, dietary restrictions and improves neurocognitive development. However, long-term follow-up is needed to evaluate whether the liver transplantation at an early age (< 1 yr) will improve the neurodevelopmental outcomes.\textsuperscript{16} Hepatocyte transplantation is considered as an alternative to orthotopic liver transplantation.\textsuperscript{19}

**Molecular diagnosis of UCD patients**

**International scenario**

A study conducted Mo et al\textsuperscript{20} suggests that patients with certain mutations exhibit more severe and early phenotype. They report that OTCB patients with I172M mutation present symptoms earlier than those with T261I or R277W mutations. Mutation analysis hence helps prenatal diagnosis and genetic counseling. This section summarizes the most important studies in this field. Most of the studies have used DNA sequencing to detect mutations. A single study has done multiplex ligation-dependant probe amplification (MLPA) method to detect mutations; this has been described under the section concerned.

**Arginase deficiency (ARGD)**

Mutation pattern in ARG1 gene in hyperargininemia in a Brazilian population has identified 8 mutations, including 5 novel ones in 16 patients. Patients with p.R308Q mutation have shown higher residual ARG1 decreased activity, but no distinguishable phenotype.\textsuperscript{21} Another study has identified patients with severe clinical presentation of arginase deficiency (ARGD).\textsuperscript{22} This study is interesting, since normally ARGD has mild presentation. A novel homozygous mutation has been identified in a Chinese patient with hyperargininemia.\textsuperscript{23}

**Citrullinemia**

Many studies have been done on the SLC25A13 gene, some of which are given below. Takahashi et al\textsuperscript{24} detected one novel and one already reported mutation in Japanese patients with citrullinemia, while Lin et al\textsuperscript{25} identified a compound heterozygote mutation citrullinemia patient from China. Tabata et al\textsuperscript{26} reported 13 novel mutations and 19 established mutations in SLC25A13 gene in citrullinemia patients from Japan, Israel, UK and Czech Republic. They reported R360X mutation in both Japanese and Caucasian population.

ASS1 gene mutations have been found in both paternal and maternal sides of a patient in a Citrullinemia Type 1 patient.\textsuperscript{27} In another study in a large cohort of 35 patients with citrullinemia, 16 novel mutations have been identified; they reported from all their previous studies, altogether 50 different mutations in 85 families with citrullinemia.\textsuperscript{28} It is found that certain mutations (G390R, R108L) of the ASS gene are associated with classical citrullinemia, whereas some other mutations (W179R, G362V) are associated with milder phenotype.\textsuperscript{29}

**CPS1 deficiency**

Seventeen mutations in patients with CPS1 deficiency have been identified in an Italian study.\textsuperscript{30} Another study in 205 patients with CPS1 deficiency over 24 yrs has identified 192 unique CPS1 gene changes; about ~10% of these mutations are found to recur in unrelated families and these affect the CpG dinucleotides. Phenotypic correlation has been found between 9 mutations and one polymorphism in CPS1 deficient patients.\textsuperscript{32}
OTC deficiency

Twenty-five distinct mutations have been described in 29 families with OTC with fourteen novel ones found in areas essential for enzyme function. A multiplex ligation-dependant probe amplification (MLPA) method has been reported to detect mutations in OTCD patients, which are undetected by other conventional methods. This technique detects deletions spanning a whole exon, large rearrangements or mutations at non-coding regions.

Three mutations (R40H, R277W and Y55D) are identified in male patients among 10 families of OTCD patients. It is also reported that paternal transmission contributes substantially to mutant allele pool and the mutant alleles associated with late-onset phenotypes are eliminated more slowly.

It is reported that the mutation Thr125Met is associated with neonatal hyperammonemia. Another study has identified 9 patients with R40H mutation and one patient with Y55D mutation in OTC gene and has reported increase in glutamine, proline, lysine, valine and methionine and decreases in serine, ornithine and arginine in these 10 patients. One of the reports has found that most of the mutations in OTC gene are point mutations and small deletions/insertions and have a “private” character (i.e., without recurrence in other families). Tuchman et al. have reported a total of 244 mutations in OTC gene, including 24 novel mutations and 13 polymorphisms), of which 42% are associated with acute neonatal hyperammonemia, 21% with late-onset disease and approximately 37% are found in heterozygous female patients. They have also reported that with conventional methods, only 80% of all mutations are detected, and the rest occur within introns or regulatory domains.

McCullough et al. have reported mutations in 157 families with OTCD. They found neonatal onset group having homogeneous clinical and biochemical phenotype, while late-onset group having extremely wide phenotype. 60% of mutations are associated exclusively with acute neonatal hyperammonemia and remaining mutations causing non-uniform phenotypes, ranging from severe disease to asymptomatic. 31% of mutations are found to occur in CpG dinucleotides. G to A and C to T transitions are the most common substitutions. In addition, it is seen that 40% of female germline mutations are in CpG dinucleotides, while the number is much smaller in male germline mutations.

Indian status

In the only study of its kind, mutation analysis from 4 patients with UCD has identified 3 patients with ASS1 mutation and one patient with OTC gene mutation. Based on DNA sequencing, the following mutations have been detected: p.Arg265Cys in homozygous state, p.Arg157His in homozygous state, p.Gly390Arg in homozygous state (all three in ASS1 gene) and a hemizygous mutation, fs352X in OTC gene (c.988_c.989 delAG). The locations of genes responsible for UCD and the common mutations seen in these genes are summarized in Table 2.

**Conclusion**

Urea cycle disorders (UCD) are an important group of inborn error of metabolism. Early diagnosis of UCD is of paramount importance in clinical practice. With early diagnosis, morbidity and mortality due to
UCD are reduced. Molecular diagnosis not only helps in reaching an early diagnosis, but also helps in prenatal diagnosis and genetic counseling. If we employ it in routine clinical practice, the cost can be brought down. We might also be able to develop multiplex PCR kits which can be used for early diagnosis, substantially reducing the cost. This review article has highlighted some of the important molecular diagnostic studies in UCD. Even though in many Western countries, molecular diagnosis is employed commonly in the diagnosis of UCD, it is not commonly practiced in India. More research is also essential to find the incidence patterns and severity of the disease in India.

References
5. D M Vasudevan, Sreekumari S & K Vaidyanathan (2013) Textbook of Biochemistry (For Medical Students) 7th edn. Publisher Jaypee Brothers, New Delhi, India.


