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Inborn Errors of Metabolism and Brain Involvement – 5 Years Experience from a Tertiary Care Center in South India

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1. Introduction

Inborn errors of metabolism (IEM) comprise a large group of more than 500 different rare genetic disorders. They arise due to mutations in genes encoding a single enzyme in metabolic pathways. Some of these disorders are very rare, whereas certain other disorders are more common. There are considerable racial and ethnic differences in the incidence pattern of these disorders. Aminoacidurias like phenylketonuria are common in the Western population; in Asian countries including India, organic acidurias like propionic acidurias, methyl malonic acidurias and maple syrup urine disease are more common. Clinical presentation of IEM is varied and it affects multiple organ systems, including CNS. Indeed CNS involvement is one of the most common presenting symptoms. The diseases can appear immediately after birth; or sometimes it may be delayed, even appearing in adult life.

In this chapter we shall describe our experience with metabolic screening in the last 5 years. This is followed by a presentation of some important case histories along with their laboratory work-up. We then go on to discuss the current global status in diagnosis and management of these diseases. It should be emphasized at the beginning itself that our laboratory (Metabolic Disorders Laboratory, Amrita Institute of Medical Science, Kochi, Kerala, S. India) is a referral center for the state of Kerala as well as the neighboring states in South India. Hence the studied population represents children who are suspected to have IEM or are high-risk individuals, or who have been referred from other hospitals in this part of India. Hence the results described do not reflect the population incidence.

If these patients are not diagnosed and treated early in life, they go on to have irreversible damage. Many body systems are affected, and the predominant damage will be to the central nervous system. The babies may develop permanent mental retardation, growth retardation, intractable seizures, cerebral palsy etc.

2. Objectives

8361 patients were screened for different metabolic disorders during the time period from September 2006 to August 2011. The screening panel included tests for aminoacidurias, organic acidurias, disorders of carbohydrate metabolism (including galactosemia,

glycosuria, fructosuria, pentosuria, mucopolysaccharidoses etc), congenital adrenal hyperplasia, pheochromocytoma, hyperhomocysteinemia, porphyrias etc.

3. Methods

Patients admitted to Amrita Institute of Medical Sciences, Kochi and other hospitals in Kerala State, South India with signs and symptoms suggestive of metabolic disorder were tested. Neurological symptoms of the patients included psychomotor delay, mental retardation, seizures, dystonia, ataxia, lethargy, coma, encephalitis, speech delay, hyperactivity etc. Non-neurological symptoms were failure to thrive, organomegaly, vomiting, skin rashes, metabolic acidosis, hyperammonemia, hypoglycemia, lactic acidosis and ketonuria.

The breakup of different tests are as follows - (1) Total number of tests - 8361 (2) Urine screened for metabolic disorders (panel including amino acids, organic acids, carbohydrates, ketone bodies etc) - 1940 (3) Amino acid screening by HPLC - 519; Organic acid screening by HPLC - 420 (4) Homocysteine estimation - 953 (5) VMA estimation - 582 (6) Porphyrias - 266 (7) 17 α hydroxy progesterone estimation (for congenital adrenal hyperplasia) - 1155 (8) Adenosine deaminase estimation - 2406 and (9) Other tests - 540 (Myoglobin, 5 HIAA, lipoprotein electrophoresis, glucose 6 phosphate dehydrogenase, homocystinuria etc). Methodologies are given under each concerned section.

4. Results

The breakup of positive cases is as follows - Aminoacidurias - 32, organic acidurias - 51 (confirmed cases), hyperhomocysteinemia - 285, pheochromocytomas and neuroblastomas - 44, elevated adenosine deaminase levels - 358 and congenital adrenal hyperplasia - 309. Further discussion is limited to aminoacidurias, organic acidurias, hyperhomocysteinemia, pheochromocytomas and neuroblastomas, since other disorders will not affect the brain.

We have divided this chapter into 3 major sections: Each of these sections will discuss the results and recent review of literature. Some rare and interesting cases are also discussed under the concerned sections. Section 1 - Aminoacidurias and organic acidurias; Section 2 - Homocysteine, Section 3 - Pheochromocytoma and neuroblastoma. Section 1 on aminoacidurias and organic acidurias is divided into 4 sub-sections: 1.1 - Maple syrup urine disease, 1.2 - Methyl malonic acidurias and propionic acidurias, 1.3 - Phenylketonuria and 1.4 - Nonketotic hyperglycinemia.

5. Section 1: Amino acidurias and organic acidurias

5.1 Materials and methods

1940 urine samples were initially screened for different aminoacidurias and organic acidurias. Simple screening tests and thin layer chromatography were used for screening. 519 samples were analyzed further for aminoacidurias and 420 samples were analyzed for organic acidurias by HPLC. 20 ml fresh urine samples and 3 mL EDTA blood samples were collected under aseptic precautions for the analysis.

5.1.1 HPLC method of amino acid analysis

Analytical Conditions were as follows - Column- LUNA C-18, Mobile phase A: 5 mM sodium phosphate buffer with pH 7.0, Mobile Phase B: 100% Acetonitrile. Gradient Elution,

Flow Rate- 1.0 ml/ min, Temperature- 40 °C, Detection- Absorption (254 nm). Samples were deproteinized and treated with phenyl isothiocyanate (PITC) and triethylamine (TEA) prior to injection (pre column derivatization).

5.1.2 HPLC method of organic acid analysis

Analytical Conditions were as follows - Column: 4.6 mm * 250 cm, Lichrocart 250-4 Lichrosorb RP -18 (Phenomenex), Mobile phase: 0.01M KH₂PO₄/H₃PO₄ (pH 3.5), Flow rate: 1 ml/min, Detection: U V 206 nm, PDA detector, Column Oven Temperature: 25 C. Urine samples were also deproteinized prior to injection.

5.1.3 Results

We detected a high incidence of aminoacidurias and organic acidurias in this population. Among organic acidurias, higher prevalence of propionic aciduria (PAA), 16 cases, and methylmalonic aciduria (MMA), 15 cases, were seen. 13 cases of maple syrup urine disease (MSUD), 1 case of isovaleric aciduria and 6 cases of alkaptonuria were detected. 5 cases of tyrosinemia, 4 cases of nonketotic hyperglycinemia (NKH) and 3 cases of phenyl ketonuria (PKU) were also confirmed. There was one case of non- PKU hyperphenylalaninemia. One patient was detected to have hypermethioninemia (484 µmol/L). Mild elevation of individual amino acids was seen in a number of cases and was not considered to be characteristic of any individual aminoaciduria. This included glycine (59 cases), alanine (44 cases), proline (17 cases), histidine (8 cases) and lysine (2 cases). This probably is a representation of increased catabolic state in these patients.

5.1.4 Review of literature

Lou et al (2011) studied 552 children at high risk by MS/MS in China and report 64 children with IEM including predominantly organic acidurias and some aminoacidurias. Niu et al (2010) did population screening on about 1.5 million Taiwanese neonates by MS/MS and found that PKU, MSUD, GA-1 and MMA were the commonest disorders. Cakmakci et al (2010) reports the use of proton MR spectroscopy and diffusion weighted MR imaging in the diagnosis of children with neurometabolic brain disorders including MSUD, Canavan disease and galactosemia. Walter et al (2009) studied cord blood in a large cohort of 24, 983 births for various IEM. Cord blood screening did not detect PKU, MSUD, argininosuccinic acidurias, MMA, glutaric aciduria type 2, MCAD deficiency etc which was diagnosed later. They conclude that cord blood screening is not recommended for IEM.

Wasant et al (2008) identified 12 cases of organic acidurias in 365 patients over 3 years from Thailand. The cases include alkaptonuria, IVA, PA, MMA, GA-I, GA-II and MCD. Shigematsu et al (2010) identified 1065 cases of IVA from 146, 000 neonates screened over three years in a Japanese population.

5.2 Section 1.1: Maple syrup urine disease

5.2.1 Case report

We report here two cases of maple syrup urine disease (MSUD). Patient 1 presented at 3 months of age with excessive irritability, abnormal posturing since birth and delayed developmental milestones. History of sibling death at Day 15 of life. The clinician reported

abnormal urine odor and clinical suspicion was MSUD, isovaleric aciduria or PKU. Laboratory analysis revealed ketonuria and metabolic acidosis. HPLC analysis of amino acid confirmed MSUD (Figure 1). Child died immediately afterwards.

Patient 2 presented at Day 12 with metabolic acidosis, abnormal urine odor, ketonuria and hepatosplenomegaly. Blood and urine studies revealed the diagnosis of MSUD. Aggressive treatment was started including branched chain amino acid restricted diet and supplementation. Patient has survived until 3 years of age, without any episode of exacerbation afterwards. Patient is on follow up. Levels of leucine, isoleucine and valine came down to normal level (Table 1).

In the case of the first patient (Patient 1), diagnosis was delayed and hence treatment could not be instituted and the baby died. But in the second case (Patient 2) diagnosis and treatment was started early in life and outcome was better. These two case studies indicate the importance of early diagnosis and treatment in MSUD.

5.2.2 Biochemical abnormalities

The name originates from the characteristic smell of urine (similar to burnt sugar or maple sugar) due to excretion of branched chain keto acids. Maple syrup urine disease (MSUD) or branched chain ketoaciduria is caused by deficiency of branched chain keto acid dehydrogenase complex (BCKAD). The basic biochemical defect is deficient decarboxylation of branched chain keto acids (BCKA). It leads to accumulation of branched chain amino acids (BCAA) Leucine, Isoleucine and Valine and corresponding branched chain α keto acids (BCKA). Five distinct phenotypes are present: Classic, Intermediate, Intermittent, Thiamine-responsive and Dihydrolipoyl dehydrogenase (E3) deficient.

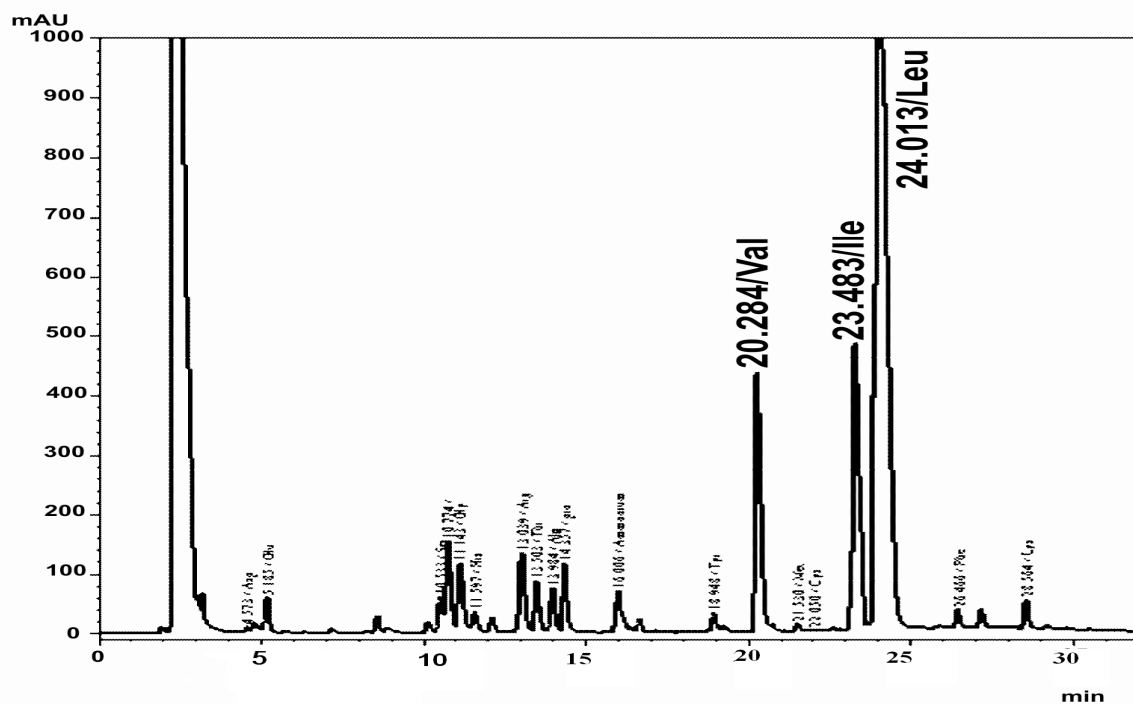


Fig. 1. Chromatogram of patient 1 (Leucine 2240 $\mu\text{mol/L}$, Valine 411 $\mu\text{mol/L}$, Isoleucine 180 $\mu\text{mol/L}$; Normal Leu <150, Val <255 and Ile <80 $\mu\text{mol/L}$)

	Valine ($\mu\text{mol/l}$)	Leucine ($\mu\text{mol/l}$)	Isoleucine ($\mu\text{mol/l}$)
11.04.2009	518	3020	437
25.07.2009	194	1708	350
15.03.2010	98	424	166
06.08.2010	135	479	69

Table 1. Serial levels of Branched chain amino acids in patient 2 diagnosed with MSUD -

Classic MSUD is the most common and is the most severe type. It has a neonatal-onset type of encephalopathy. Other types generally have onset by 2 years. BCAA, generally Leucine, is elevated in blood and urine. Presence of alloisoleucine is diagnostic. It has autosomal recessive inheritance. Worldwide frequency is 1 in 1,85,000.

BCAA comprise 35% of indispensable amino acids in muscles. Majority of untreated classic patients die within the early months of life from recurrent metabolic crises and neurologic deterioration. Treatment involves long-term dietary management and aggressive intervention during acute metabolic decompensation. Age at diagnosis and subsequent metabolic control are the most important determinants of long-term control. Patients in whom treatment is initiated after 10 days of age rarely achieve normal intellect.

Disease starts in the first week of life. It is characterized by convulsions, severe mental retardation, vomiting, acidosis, coma and death within the first year of life. Urine contains branched chain keto acids, valine, leucine and isoleucine. Rothera's test is positive, but unlike in cases of ketoacidosis, even boiled and cooled urine will give the test. Diagnosis depends on enzyme analysis in cells. Diagnosis should be done prior to 1 week after birth. Giving a diet low in branched chain amino acids. Mild variant is called intermittent branched chain ketonuria. This will respond to high doses of thiamine. This is because the decarboxylation of the BCKA requires thiamine. Liver transplantation has been successfully tried in some cases of MSUD.

5.2.3 Review of literature

Chen et al (2010) has reviewed 15 cases of MSUD from China and they suggest that early diagnosis and treatment can help prevent neurologic signs. Pangkanon et al (2008) report 13 cases of MSUD in Thai infants. All patients had neurological manifestations and psychomotor retardation. Lee et al (2008) report 47 Filipino patients with MSUD which is the commonest IEM in Philippines. They report that clinical outcome is poor in their series of patients.

Barschak et al (2009) report MSUD is associated with lipid peroxidation. They also report reduced amino acids methionine and tryptophan, which are amino acids with antioxidant activity. Mescka et al (2011) report protective effect of carnitine against oxidative stress induced by MSUD.

Ribeiro et al (2008) report that the major metabolites accumulating in MSUD disturb brain aerobic metabolism by compromising the citric acid cycle and the electron flow through the respiratory chain. They hypothesize that this might explain the neurological features in MSUD.

Brunetti-Pierri et al (2011) report successful use of phenylbutyrate in bringing down branched chain amino acid levels in MSUD. Shellmer et al (2011) studied 14 patients who received liver transplantation for MSUD and found that liver transplantation reduced further CNS damage in these patients. Strauss et al ((2010) has suggested novel therapeutic modalities in the management of MSUD based on their experience in treating 79 patients over 20 years.

Zinnanti et al (2009) report that rapid brain leucine accumulation displaces other essential amino acids resulting in neurotransmitter depletion and disruption of normal brain growth and development in mouse model. They also report that administration of norleucine reduces branched chain amino acid accumulation in brain, blood and milk. Norleucine also substantially delayed encephalopathy in intermediate type MSUD. They conclude that brain damage in MSUD might be due to two factors - (1) Neurotransmitter deficiencies and growth restriction associated with BCAA accumulation, and (2) Energy deprivation through Krebs' cycle disruption associated with BCAA accumulation.

Ibarra-Gonzalez et al (2007) report increased mortality and disabilities, especially neurological, in a cohort of 36 patients with MSUD from Mexico. Kowalik et al (2007) report deficiency of iron, zinc, copper, Vitamin B1, B2, niacin and Vitamin C in treated MSUD patients. Wajner et al (2007) report that BCAA inhibited Na-K ATPase pump in the brain and alanine prevented this inhibition. They conclude that this mechanism might contribute to neurological damage in MSUD, as Na-K ATPase pump is a critical enzyme for normal brain development and functioning. Bridi et al (2005) also report that alpha keto acids accumulating in MSUD stimulate lipid peroxidation and reduce antioxidant defense in cerebral cortex.

There are a large number of studies which report on CT and MR scans of the brain in MSUD [Cakmakci et al (2010), Tu et al (2005), Sener (2004), Schonberger et al (2004) and others]. Bindu et al (2007) have described neuroradiological findings in 3 patients with intermediate MSUD from South India.

5.3 Section 1.2: Methylmalonic aciduria and propionic aciduria

5.3.1 Results

We have detected 16 cases of propionic acidurias and 15 cases of methyl malonic acidurias in our study. Most of these patients had neurological manifestations and presented with metabolic acidosis and/or hyperammonemia. 60% of patients had neurological abnormalities including psychomotor delay, mental retardation, seizures, dystonia, ataxia, lethargy, extrapyramidal symptoms, encephalopathy, coma, visual deficiency, speech delay etc.



Fig. 2. Screening test for methyl malonic aciduria (Emerald green is positive test; other test tube is control urine sample)

Abnormal MRI findings were found in 11 patients including macrocephaly, cerebral atrophy and cerebral edema. Further details of this work can be seen in our paper (Narayanan et al, 2011; Vaidyanathan et al, 2011). Figure 2 gives a screening test for methyl malonic acid.

5.3.2 Biochemical abnormalities

Propionyl CoA is primarily converted to methyl malonyl CoA, which is subsequently converted to Succinyl CoA. Enzymes involved are propionyl CoA carboxylase, methyl malonyl CoA racemase and methyl malonyl CoA mutase. Biotin is needed for first and cobalamin for the third enzyme. Propionyl CoA carboxylase has two non-identical sub-units α and β , biotin binds to α sub-units, located on chromosomes 13 and 3 respectively. Methyl malonyl CoA mutase has 2 identical α sub-units, located on chromosome 6. Holocarboxylase deficiency and biotinidase deficiency are known. Other known disorder is multiple carboxylase deficiency. PCC deficiency leads to propionic acidemia, and elevated 3 hydroxy propionate, methyl citrate, tiglyl Glycine, and unusual ketone bodies in urine. Severe metabolic ketoacidosis in neonatal period is seen. Alkali therapy and protein restriction are needed.

Inherited deficiency of the mutase enzyme or abnormalities in cobalamin can result in methyl malonic aciduria. Neonatal or infantile metabolic ketoacidosis are the hallmarks. Patients with abnormal binding ability of enzyme to cobalamin, cannot be treated by cobalamin therapy. These cases may be treated with dietary protein restriction and antibiotic therapy. Other patients respond to cobalamin or hydroxycobalamin therapy. This can be used in combination with dietary protein restriction. Therapy has been found to reduce methyl malonate levels. Mutations leading to impaired adenosyl cobalamin and methyl cobalamin and deficient activity of methyl malonyl CoA mutase and N5 methyl tetrahydro folate reductase. Homocysteine methyl transferase have methyl malonic aciduria combined with homocystinuria. Features include failure to thrive, developmental retardation, megaloblastic anemia and macrocytosis. Therapy includes protein restriction, pharmacological doses of hydroxocobalamin and betaine supplementation.

Both disorders are inherited as autosomal recessive disorders. Prenatal diagnosis is possible by enzyme assays on chorionic villus biopsy or cultured amniotic cells and chemical determinations on amniotic fluid or maternal urine.

5.3.3 Review of literature

Liu et al (2010) reports 24 mutations in the MMACHC gene to be responsible for cblC type of combined methyl malonic aciduria and homocystinuria in 79 unrelated Chinese patients. 5 mutations are responsible for 80% of cases and suggest a role for mutation detection in early diagnosis. Cosson et al (2009) reports on long term outcome of 30 French patients with methyl malonic acidurias. 15 patients had neonatal onset, 13 had severe neurological involvement, 14 had chronic renal failure and 5 died during a metabolic crisis. Patients with a mut(0) phenotype had a severe phenotype and early and more severe CRF than patients with mut-/cblA phenotype.

Chandler et al (2007) reports successful use of adenoviral mediated gene therapy for methylmalonic aciduria in murine models and human patients with MUT gene mutation. Yang et al (2006) and other authors report that in China methylmalonic acidurias is more commonly associated with homocysteinemia. Filippi et al (2010) describes the use of N -

carbonyl glutamate in the emergency management of hyperammonemia in neonatal acute onset propionic aciduria and methylmalonic aciduria. Perez et al (2009) describes pseudoexon exclusion by antisense therapy in MMA. Haas et al (2009) report that Coenzyme Q (10) is significantly decreased in MMA patients.

Wajner et al (2009) identified 34 patients with MMA and 18 patients with PA from Brazil in 15 years. Zwickler et al (2008) reviews MMA patients in 14 centers in Germany and outlines the management principles. Most centers used hydroxocobalamin or cyanocobalamin for cobalamin-responsive patients while cobalamin – nonresponsive patients are supplemented with carnitine. Intestinal decontamination by antibiotic therapy, D-A-CH or Dewey recommendations for protein therapy and precursor-free amino acid supplements were used by most centers. Zhang et al (2007) studied the clinical picture of 96 patients with MMA over a 10 year period. Most of the patients had neurological abnormalities including developmental delay, seizures, psychomotor degeneration and motor disorders. A significant proportion of patients had MMA along with homocysteinemia.

Longo et al (2005) describes neurological abnormalities in MMA patients detected by MRI and 1H-MRS. Burlina et al (2003) and Rossi et al (2001) also describe neurological damage in MMA. Bodamer et al (2005) studied creatine metabolism in MMA patients and found that guanidinoacetate is elevated. They suggest that guanidinoacetate may be responsible for neurotoxicity in MMA. Vara et al (2011) discusses the importance of liver transplantation in propionic aciduria (PA) patients. Liver transplantation reduces the risk of metabolic decompensation and improves the quality of life. Romano et al (2010) report that cardiomyopathy in PA is reversible with liver transplantation.

Ah Mew et al (2010) also reports that NCG reduces ammonia and glutamine and induces ureagenesis in PA. Schwahn et al (2010) also reports of NCG in PA. Chandler et al (2011) describes the use of gene therapy using adeno associated virus (AAV8) for the treatment of PA. Ribas et al (2010) report that PA and MMA induce DNA damage and L – carnitine prevents this damage. Haberlandt et al (2009) report seizures in 9 patients with PA and hypothesize that some intermediates might be involved in the pathogenesis of epilepsy. Rigo et al (2006) suggests the involvement of NMDA receptors in the pathogenesis of neurological manifestations.

de Keyzer et al (2009) report multiple OXPHOS deficiency in liver, kidney, heart and skeletal muscle in patients with MMA and PA. Desviat et al (2009) report high frequency of large gene deletions in PA patients. This finding underscores the need for gene dosage analysis in addition to mutation testing in PA patients. Perez Cerda et al (2004) report the utility of prenatal diagnosis by molecular methods in 19 unrelated families with PA.

5.4 Section 1.3: Phenylketonuria (PKU)

5.4.1 Case report

We hereby report two patients with PKU. Patient 3 is a 21 year old woman with sub-normal intelligence and suspected to have phenylketonuria, though not confirmed previously (Phenylalanine level – 1427 $\mu\text{mol/L}$, Normal <65 $\mu\text{mol/L}$). Patient 4 is 19 years old and is the sibling of Patient 2 (Phenylalanine level 1177 $\mu\text{mol/L}$). She also had sub-normal intelligence. Both patients had pleasant social manners. At the time of presentation, Patient 3

was pregnant and had hence sought advice. Three months after presentation, Patient 4 also became pregnant. Both patients were confirmed to have phenylketonuria by urine and blood tests for phenylalanine. Phenylalanine restricted diet was advised, but compliance was not satisfactory and phenylalanine levels remained above 1000 $\mu\text{mol/L}$. Patient 3 delivered and child suffered from clinical and laboratory signs of maternal hyperphenylalaninemia. Child died in the immediate post natal period. The child of the other patient died in utero.

5.4.2 Biochemical abnormalities

Hyperphenylalaninemias are due to disorders of phenylalanine hydroxylation reaction. The minimum requirements for phenylalanine metabolism to occur are the enzyme phenylalanine hydroxylase (PAH), molecular oxygen (O_2), L-Phenyl Alanine and tetrahydrobiopterin (BH_4). Other components include dihydrobiopterin (DHPR), reduced pyridine nucleotide, 4 α carbinolamine dehydratase (for BH_4 recycling), GTP cyclohydrolase (GTP - CH) and 6 pyruvoyl tetrahydropterin synthase (6 PTS). Hyperphenylalaninemia is defined as Phenylalanine levels above 120 μM (2 mg/dl). Normal plasma level of phenylalanine is $58 \pm 15 \mu\text{M}$.

Phenylalanine cannot be converted to tyrosine. So phenylalanine accumulates. Phenylalanine level in blood is elevated. So alternate minor pathways are opened. Phenyl ketone (phenyl pyruvate), phenyl lactate and phenyl acetate are excreted in urine. Phenyl pyruvate inhibits pyruvate decarboxylase enzyme in brain, but not in liver. Hence myelin formation defects and mental retardation are seen. Brain effects are due to phenylalanine and its metabolites (phenyl ketones, namely Pyruvate, lactate, acetate, acetyl glutamine and ethyl amine) that accumulate via alternate pathway. Myelination and protein synthesis are affected and there is deficient neurotransmitter supply. Peculiarities of gait, stance and sitting posture are additional features. Brain calcification may be seen in DHPR deficient type.

Phenylketonuria is well known for the neurological manifestations. The classical PKU child is mentally retarded with an IQ of 50. About 20% inmates of psychiatric hospitals may have PKU. Agitation, hyperactivity, tremors and convulsions are often manifested. This may be because phenylalanine interferes with neurotransmitter synthesis. The child often has hypopigmentation, explained by the decreased level of tyrosine. Phenyl lactic acid in sweat may lead to mousy body odor.

5.4.3 Maternal hyperphenylalaninemia

Female child, on growing to adulthood may become pregnant (maternal hyperphenylalaninemia). Then again special diet is to be given, because the increased phenylalanine level will affect the brain development of the fetus. Maternal hyperphenylalaninemia (PKU embryo-fetopathy) cause embryopathy/ fetopathy comprising impaired growth, congenital cardiac malformations, microcephaly and mental retardation in the embryo/fetus. Fetal phenylalanine level is 1.5 - 2 fold higher than maternal blood level. Further fetal blood brain barrier concentrates phenylalanine to another 2-4 fold. Intra-neuronal phenylalanine level of 600 μmol interferes with brain development. Phenylalanine restricted diet should be started at least 3 months prior to planned pregnancy. The level in mother to be maintained at 60-180 $\mu\text{mol/L}$. Linoleic and linolenic acid supplements should be maintained at high level.

5.4.4 Review of literature

Oddason et al (2011) report 27 patients diagnosed with PKU in Iceland since 1947. Classical PKU is the commonest type. Macdonald et al (2011) in a study from UK report that PKU patients, especially older, are not fully compliant with treatment and hence have higher than acceptable phenylalanine levels. At the same time, van Rijn et al (2011) report that well-controlled adult PKU patients can tolerate larger dietary variations in phenylalanine levels. ten Hoedt et al (2011) report that high phenylalanine levels can directly affect mood and sustained attention in adult PKU patients (Randomized, double-blind, placebo-controlled, crossover trial).

Ribas et al (2011) suggest that oxidative stress may play a role in pathogenesis of PKU. Sanayama et al (2011) provide experimental evidence for the same and report that oxidative stress status is closely linked with phenylalanine levels. Sitta et al (2011) report that administration of L-carnitine and selenium can reduce oxidative stress in PKU patients.

Hanley (2011) in a review states that “non-PKU mild hyperphenylalaninemia” (MHP) also might have neuropsychological function deficits and therefore may need treatment with tetrahydrobiopterin and/or phenyl alanine restricted diet. Campistol et al (2011) discusses on the extent of neuro-cognitive dysfunction in mild PKU (mPKU) and conclude that further studies are needed on mPKU to clearly answer this question. van Spronsen (2011) addresses the question on treatment of mPKU and reaches similar conclusion.

Vernon et al (2010), Trefz et al (2010), Harding (2010), Burton et al (2010), Somaraju & Merrin (2010) and others report that saptopterin hydrochloride, a synthetic analog of tetrahydrobiopterin, is a promising new drug in the treatment of PKU. This modality of treatment is especially important considering the report by Enns et al (2010) who state that PKU treated with diet alone has sub-optimal outcome with relation to neurocognitive, psychological and physical parameters. Lee et al (2009) and others however, report better outcome with dietary treatment alone. Blau et al (2010) reports on the management practices in PKU in 165 PKU centers in 23 European countries. They emphasize that treatment recommendations vary tremendously in different countries and hence there is an urgent need to pool long-term data in registries to generate evidence based international guidelines. Ahring et al (2009) and van Spronsen (2009) give similar reports.

Rocha and Martel (2009) report the use of large neutral amino acids in the treatment of PKU. The same carrier transports phenylalanine as well as large neutral amino acids into the brain; hence their use diminishes toxicity due to phenylalanine. Weigel et al (2008) report low free carnitine levels in PKU patients given low phenylalanine diet. They suggest that carnitine level should be monitored in PKU patients. Sitta et al (2009) reach similar conclusions. A study by Maillot et al (2008) reports on the importance of maintaining blood phenylalanine during pregnancy. They conclude that maintenance of maternal blood phenylalanine level within the target range predicts good offspring outcomes; and further suggests that variations even within that range should be avoided.

5.5 Section 1.4: Nonketotic hyperglycinemia

5.5.1 Case report

We report here one male baby (Patient 5) with intractable seizures who was 7 days old. All antiepileptic drugs were tried without any response. The parents were complaining about medical negligence. HPLC analysis of amino acid revealed nonketotic hyperglycinemia (NKH) (Figure 3). Even though the child could not be revived; this case shows the

importance of workup for inborn errors of metabolism to reach a diagnosis. In this case, the diagnosis was important for the doctor to counsel the parents appropriately.

5.5.2 Biochemical abnormalities

It is due to defect in **glycine cleavage system**. Glycine level is increased in blood, urine and CSF. Severe mental retardation and seizures are seen. There is no effective management. Large quantities of Glycine accumulate in all body tissues including CNS. Diagnosis is established by CSF: plasma Glycine concentration >0.08. Patients have a neonatal phenotype and present in the first few days of life with lethargy, hypotonia and myoclonic jerks and progressing to apnea and death. Surviving infants have intractable seizures and profound mental retardation. Later-onset children have progressive spastic diplegia and optic atrophy, but mental retardation and seizures may not be seen. Transient NKH is also reported. It is inherited as an autosomal recessive disorder. In neonatal NKH CSF: plasma ratio may be 0.09-0.25 and in atypical NKH it is 0.09 – 0.10. Normal ratio is 0.012 – 0.040.

5.5.3 Review of literature

Aburahma et al (2011) report that elevated CSF/plasma glycine level is encountered in a variety of clinical conditions and hence cannot be considered to be pathognomonic of nonketotic hyperglycinemia (NKH). This report is significant because NKH is a disease with very bad prognosis. Lang et al (2008) discusses the difficulties of diagnosing transient NKH.

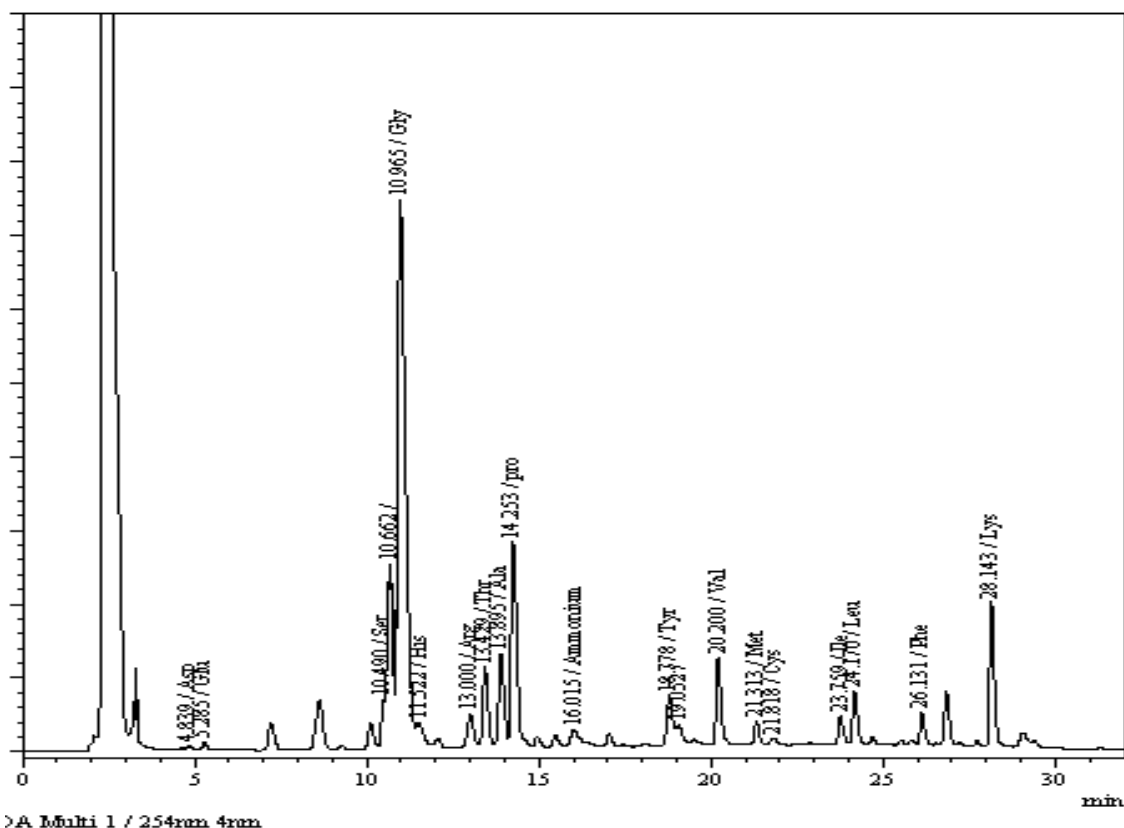


Fig. 3. Chromatogram of patient 5 showing elevated glycine levels (Glycine 1311 $\mu\text{mol/L}$, Normal Glycine < 275 $\mu\text{mol/L}$)

Leipnitz et al (2009) report lipid peroxidation and reduced antioxidant levels in NKH. Kanno et al (2007) reviews the genetic causes of NKH. NKH can be caused by genes in the glycine cleavage system, including GLDC, AMT and GCSH. They report significant number of GLDC mutations by MLPA (multiple ligation-dependent probe amplification) analysis. Conter et al (2006) and Kure et al (2006) also report significant number of mutations in these genes.

Tan et al (2007) report that currently tandem mass spectrometry employed for newborn screening does not identify NKH without significant error rate. Raghavendra et al (2007) and others report significant neurological abnormalities in patients with NKH. Generally NKH is a disease refractory to treatment. A number of authors report the use of sodium benzoate and dextromethrophan in the treatment of NKH, some of them with and others without any beneficial effect.

6. Section 2: Hyperhomocysteinemia

6.1 Materials and methods

Homocysteine estimation was done by ELISA method (BioRad Laboratories Inc.). 5 ml blood was drawn from the patients. 953 patients were analyzed during this 5 year period, 110 patients were from the Department of Cardiology, 656 from the Department of Neurology and the remaining from other departments.

6.2 Results

285 patients had elevated homocysteine levels. 226 cases had hyperhomocysteinemia from the Department of Neurology (226/656, 34.5%), 31 had hyperhomocysteinemia from the Department of Cardiology (31/110, 28.2%), and the remaining were other cases like peripheral artery disease, deep vein thrombosis etc. Neurological disorders included different types of stroke including medullary stroke, ischemic stroke, young stroke, transient ischemic attack, sagittal sinus thrombosis, lacunar thalamic stroke and recurrent stroke. All patients with hyperhomocysteinemia from the Department of Cardiology were suffering from Coronary artery disease (CAD).

6.3 Biochemical abnormalities

Normal homocysteine level in blood is 5-15 $\mu\text{mol/L}$. In diseases, it may be increased to 50 to 100 times. Moderate increase is seen in aged persons, vitamin B12 or B6 deficiency, tobacco smokers, alcoholics and in hypothyroidism. Substantial increase is noticed in congenital enzyme deficiencies. Large amounts of homocysteine are excreted in urine. In plasma, homocysteine (with -SH group) and homocysteine (disulfide, -S-S- group) exist. Both of them are absent in normal urine; but if present, it will be the homocysteine (disulfide) form. If homocysteine level in blood is increased, there is increased risk for coronary artery diseases. Homocystinuria/ hyperhomocysteinemia may be due to many causes. These include impaired activity of CBS (genetic CBS deficiency, INH therapy), methionine synthase defect, MTHFR defect, impaired metabolism of vitamin B12, renal insufficiency, pyridoxine and folate deficiency etc.

6.4 Review of literature

Hyperhomocysteinemia is associated with a number of diseases including coronary artery diseases, stroke, retinal vein thrombosis, diabetic peripheral neuropathy, schizophrenia, preeclampsia, chronic pancreatitis etc. Herrmann and Obeid (2011) have reviewed the role of hyperhomocysteinemia in neurodegenerative diseases like Alzheimer's disease, vascular dementia, cognitive impairment and stroke. Damelan et al (2010) report hyperhomocysteinemia is ischemic stroke patients from France. Valentino et al ((2010) report elevated blood and CSF levels of homocysteine in amyotrophic lateral sclerosis (ALS).

Snieszawska et al (2011) report that antiepileptic drug (AED) treatment in epileptics leads to increase in homocysteine and asymmetric dimethyl arginine (ADMA) levels. Greater increase in homocysteine is observed in patients with MTHFR CT (C677T) and MTHFD1 GG (G1958A) polymorphisms. Linnebank et al (2011) report reduced serum Vitamin B12 and folate levels on treatment with AED. Zhuo et al (2010) report that normalization of homocysteine values in rat models with hyperhomocysteinemia resulted in improvement of cognitive defects and brain amyloidosis.

Paoli et al (2010) report that protein N homocysteinylation induces the formation of toxic amyloid like amyloid protofibrils. The authors hypothesize that this could be responsible for pathophysiology of hyperhomocysteinemia. da Cunha et al (2010) report increase in inflammatory markers in brain and blood of mice after acute homocysteine administration. This study suggests that inflammation may be at least partially responsible for neurological and cardiovascular response of homocysteine. Green et al (2010) report that homocysteine lowering vitamins do not lower S adenosyl homocysteine levels in older people. They suggest that S adenosyl homocysteine might be a better indicator for vascular events. They hypothesize that this might explain the lack of clinical benefit of B vitamins in some patients with hyperhomocysteinemia. Almawi et al (2009) and others have investigated the role of MTHFR C677T polymorphism and high homocysteine levels in patients with stroke. Dutta et al (2009) report mild increase in serum homocysteine levels in Indian patients with idiopathic mental retardation.

7. Section 3: Pheochromocytoma, neuroblastoma

7.1 Materials and methods

VMA estimation was done using column method (BioRad VMA by column test). 24 hr urine samples were collected following dietary restrictions for 3 days. Samples were collected in 6N HCl and stored in the refrigerator during collection time. If the pH was more than 3.5, the samples were rejected. The analysis was done following manufacturer's guidelines. 582 samples were collected during this time period.

7.2 Results

44 patients had elevated VMA levels. This included 24 cases of pheochromocytoma, 15 cases of neuroblastoma and 5 cases of paraganglioma. The analysis correlated positively with histopathology studies. All patients with pheochromocytoma and paraganglioma were above 10 years of age; whereas 60% cases of neuroblastoma cases were below 10 years of age. The sensitivity and specificity of our results were 88% and 86% respectively. All neuroblastoma cases showed elevated VMA level.

7.3 Biochemical abnormalities

Catecholamines are important biological compounds, essential to maintain the proper functioning of the body. Secretion of excessive catecholamines can produce stress, palpitation, paroxysmal hypertension, congestive heart failure, thyroid hormone deficiency and arrhythmias. Measurement of catecholamines and its metabolites is primarily used in the diagnosis of Neuroendocrine tumors; Neuroblastomas, Pheochromocytomas and paragangliomas. Most common metabolites of catecholamines are Vanillylmandelic acid (VMA), urinary free catecholamines, metanephrine, normetanephrine, homovanillic acid (HVA), plasma catecholamines, plasma metanephrines and chromogranin A. Most of the metabolites are measured by spectrophotometric assays, nowadays replaced by HPLC and mass spectrometry. Vanillylmandelic acid (VMA) is a major catecholamine metabolite formed by the actions of catechol-O-methyl transferase and MAO. VMA is excreted by kidney and represents 40%-50% urinary excretory product of norepinephrine and epinephrine. Norepinephrine is the major source of VMA.

Neuroblastomas are the most common solid extra-cranial tumors in children, and account for 7-10 % of all tumors. In about 90% of cases of neuroblastoma, elevated levels of catecholamines or its metabolites are found in the urine or blood. Pheochromocytomas are chromaffin-cell tumors; 80-85 % arises from the adrenal medulla and 15-20 % arises from extra-adrenal chromaffin tissues (paragangliomas). They are characterized by excessive production of catecholamines. If not diagnosed or if left untreated, the excessive secretion of catecholamines by these tumors can have devastating consequences. Paraganglioma is a rare neuroendocrine tumor that arises from extraadrenal sympathochromaffin tissue, usually in the abdomen. 10% of the catecholamine producing tumors are paragangliomas that develop in head, neck, thorax and abdomen. About 97% are benign and 3% metastatic.

7.4 Review of literature

Elevated urinary catecholamines have been described in 90 - 95 % of patients with neuroblastomas [Smith et al (2010)]. Studies suggest that dopamine nervous systems are involved in the pathogenesis of autistic disorder. Quantification of urine homovanillic acid (HVA) and vanillylmandelic acid (VMA) by GC/MS are very important in the study of dopamine metabolism in autistic children [Kaluzna-Czaplinska et al (2010)]. Allenbrand and Garg (2010) also report quantification of urine HVA and VMA by GC/MS. Li et al (2010) describe that the ratio of HVA/VMA ratio is useful as a disease marker in neuroblastomas and pheochromocytomas. Aydin et al (2010) report that VMA is a poor prognostic factor in patients with neuroblastoma.

Hickman et al (2009) report that in patients with pheochromocytoma, plasma free metanephrines displayed superior diagnostic sensitivity and specificity compared with other biochemical markers of catecholamine output and metabolism. Boyle et al (2007) came to a similar conclusion. Lionetto et al (2008) describe an HPLC-tandem mass spectrometric method for the simultaneous quantification of VMA, HVA and 5-HIAA in human urine.

8. Conclusions

We have detected a high incidence of metabolic disorders in our population. The commonest disorders detected were organic acidurias, congenital adrenal hyperplasia and

hyperhomocysteinemia. Aminoacidurias like PKU are rare; whereas organic acidurias (PAA, MMA and MSUD) are more common. Prompt identification and treatment is important and early diagnosis helps to institute treatment measures which prevents further morbidity and reduces mortality rates.

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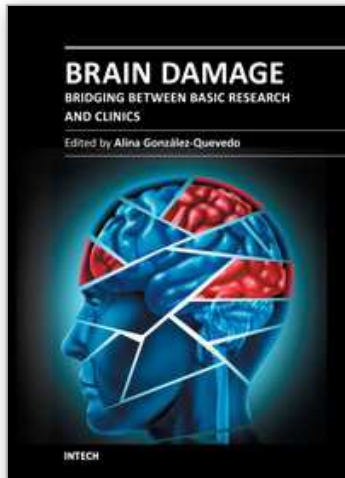
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"Brain Damage - Bridging Between Basic Research and Clinics" represents a collection of papers in an attempt to provide an up-to-date approach to the fascinating topic of brain damage in different pathological situations, combining the authors' personal experiences with current knowledge in this field. In general, the necessary link between basic and clinical neurosciences is highlighted, as it is through this interaction that the theoretical understanding of the pathophysiological mechanisms can be successfully translated into better ways to diagnose, treat and prevent the catastrophic events that occur when the brain suffers from external or internal noxious events. The book spans different aspects of brain injury, starting from damage occurring in the fetal and child brain, followed by different neurodegenerative processes. Attention is also focused on the negative effects of drug addictions and sleep deprivation on the brain, as well as on the early assessment of brain injury for preventive strategies employing sensitive biomarkers.

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