Investigation of metabolic encephalopathy

Encephalopathy may be a presenting sign in a wide range of medical conditions.

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Encephalopathy may be a presenting sign in a wide range of medical conditions. This review focuses only on the diagnosis and initial management of those inherited metabolic diseases (IMDs) prevalent in South Africa that may present with encephalopathy in childhood. Metabolic encephalopathy is a medical emergency, and appropriate empirical intervention can often improve outcome, provided a timely working diagnosis is made based on clinical suspicion and targeted laboratory investigations.

Clinicians should be aware of disorders that are more likely to occur within the population they are treating. A family history, evidence of previous siblings with similar presentations or consanguinity should always be sought.

Introduction

Most IMDs are caused by autosomal recessive (AR) single enzyme deficiencies. Some follow an X-linked recessive pattern, affecting mainly boys, or in the case of some mitochondrial disorders, a strictly maternally inherited pattern. The spectrum and frequency of IMDs in a population are strongly influenced by consanguinity and the prevalence of founder mutations. Clinicians should be aware of disorders that are more likely to occur within the population they are treating. A family history, evidence of previous siblings with similar presentations or consanguinity should always be sought.

Genetic population studies have shown, for example, that high carrier frequencies of single mutations in the South African black population are responsible for most cases of glutaric aciduria type 1 (GA1), galactosaemia and cystinosis.[1-3] Furthermore, our experience has shown that whereas propionic acidaemia (PA) is frequently encountered across all population groups, isovaleric acidaemia (IVA) is mostly confined to Afrikaners. Furthermore, common European disorders such as phenylketonuria and medium chain acyl dehydrogenase deficiency (MCADD) are relatively uncommon in South Africa.

By far the most common of the urea cycle defects is the X-linked recessive disorder, ornithine transcarbamylase deficiency (OTC). X-linked recessive disorders usually arise as novel maternal germ-line mutations and therefore occur with similar frequencies worldwide. Mitochondrial cytopathies can be caused either by AR deficiency of many nuclear encoded genes or by mutations in the smaller mitochondrial genome. Because mitochondria derive exclusively from the maternal oocyte, the latter disorders are only passed from a mother to her children and are perpetuated through her daughters.

Table 1 presents a series of selected IMDs diagnosed at the Red Cross Children's Hospital Metabolic Disease Laboratory over the preceding 6 years that may potentially present with metabolic encephalopathy. These cases represent over 60% of all IMDs diagnosed in this period and the majority fall into 3 main categories, namely the organic acidaemias (OAs), the urea cycle defects (UCDs) and the mitochondrial cytopathies. Not included in this series are those cases where encephalopathy may be secondary to failure of other organs such as hepatorenal failure in tyrosinaemia type 1 and galactosaemia, adrenal failure in adrenoleukodystrophy (X-ALD) or hypoglycaemia in glycogen storage disease type 1.

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Organic acidaemias

The OAs are largely represented by deficiencies of enzymes involved in the catabolism of amino and fatty acids for fuel. During catabolism, organic acid intermediates accumulate and clinical decompensation is mostly ascribed to the inherent toxicity of these intermediates or the fact that they may disrupt other critical metabolic pathways. Diagnostic metabolites can be measured in blood or in urine and, together with elevated precursor amino or fatty acids, are used to make a diagnosis. Typically patients are far more likely to decompensate when the affected pathway is stressed during major catabolic episodes as seen with inter-current infections, or the early neonatal transition to extra-uterine life, or if the child is fed the compounds that they are unable to metabolise. Typically,

Table 1. Confirmed IMD cases associated with metabolic encephalopathy diagnosed at Red Cross Children's Hospital Metabolic Disease Laboratory, 2006 - 2012

Name of disorder	Number of cases
Glutaric aciduria type 1 (GA1)*	23
Mitochondrial cytopathies (all)	11
Propionic acidaemia (PA)*	11
Urea cycle defects (all)	7
Non-ketotic hyperglycinaemia (NKH)	5
Pyruvate dehydrogenase deficiency (PDH)*	5
Ketone utilisation defects (all)*	4
Fatty acid oxidation defects (FAODs – all)*	4
Biotinidase deficiency*	3
Isovaleric acidaemia*	3
Multiple acyl dehydrogenase deficiency*	3
Glutathione synthetase deficiency*	2
Methylmalonic acidaemia (MMA)*	2
Maple syrup urine disease (MSUD)*	2
Molybdenum cofactor deficiency	2
l-2-hydroxyglutaric aciduria*	1
HMG-CoA-lyase deficiency*	1
Malonyl-CoA decarboxylase deficiency*	1

*Organic acidaemias.

the resultant metabolic derangements, all of which can contribute to depressed neurological function, are the following:

- high anion-gap metabolic acidosis due to the accumulated organic acids themselves
- hyperammonaemia due to secondary disruption of the urea cycle
- hypoglycaemia due to secondary inhibition of hepatic gluconeogenesis
- lactataemia due to failed gluconeogenesis
- ketonaemia where intermediates may feed into ketone biosynthesis.

It is important to note that in some disorders such as PA and biotinidase deficiency, all the above metabolic derangements may be present. Others, such as the fatty acid oxidation defects (FAODs), typically only present with the first 3 and maple syrup urine disease (MSUD) usually presents only with a severe anion-gap acidosis. Further, clinical clues to some OAs may be the distinct smell of caramelised sugar in MSUD and the offensive goat-like cheesy odour of isocaproate that accumulates in IVA and multiple acyl dehydrogenase deficiency.

The classic neonatal presentation of OTC involves an apparently healthy male neonate who develops feeding difficulties, lethargy and hypersomnolence within the early neonatal period that rapidly progresses to life-threatening hyperammonaemic encephalopathy.

Glutaric aciduria type 1 is the most common OA in South Africa and is caused by AR deficiency of glutaryl-CoA dehydrogenase (GCDH), a critical enzyme in the infantile neuronal lysine catabolic pathway.[1] Due to a relatively large size and limited hepatic glucose production, the infantile brain relies heavily on ketones and amino acids like lysine as a fuel source during times of catabolic stress. Children with GA1 are born and develop normally, until they typically present during infancy with hypotonia and encephalopathy associated with an episode of catabolic stress. During these episodes, massive levels of the toxic lysine metabolites, 3-OH glutarate and glutarate accumulate in the brain, causing disruption of neuronal mitochondrial function and irreversible damage to the basal ganglia.

Children go on to develop a severely debilitating form of choreo-athetoid cerebral palsy, often with retention of a normal intellect. In addition, GA1 can be associated with microencephalic-macrocephaly, with a high risk of repeated intracranial and even retinal haemorrhages. The clinical and radiological profile is easily confused with repeated blunt non-accidental head injury.[4] If GA1 is diagnosed prior to the first acute episode, the outcome can be significantly altered by dietary lysine restriction, carnitine supplementation, and

most importantly an 'emergency regimen' consisting of the provision of liberal glucose during periods of catabolic stress.[5] After 24 months, the risk of acute episodes drops dramatically as the liver is able to supply brain glucose requirements. Appropriately treated children may have an entirely normal outcome.[3]

GA1 is easily diagnosed by analysis of urine organic acids and confirmed by GCDH mutation analysis. In South Africa, 1 in 35 to 1 in 50 black people are carriers for the same A293T mutation in the GCDH gene and at least 60 children are estimated to be born with this disorder each year – most of them never receive a correct diagnosis![1]

Urea cycle defects

The UCDs as a group constitute approximately 12% of emergency admissions due to IMDs.[6] They all involve deficiencies of enzymes or transporters involved in the hepatic cyclical conversion of neuro-toxic ammonia, to urea. All are exacerbated by increased protein intake or catabolic stress, in much the same manner as the OAs. Isolated hyperammonaemia is the hallmark of the UCDs and clinical presentations vary from acute encephalopathy with brain oedema to milder symptoms such as learning difficulties, drowsiness and avoidance of high-protein foods. Proximal defects in the urea cycle are generally associated with higher ammonia levels.[7]

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Table 2. Laboratory investigation of suspected metabolic encephalopathy

Laboratory investigations Tier 1	Sample requirements	Rationale
Arterial blood gas plus plasma Na^+, K^+ and Cl^- for anion gap calculation	Capped heparin blood on ice, plus 1 ml heparin tube	High anion gap acidosis in OAs, respiratory alkalosis in UCDs
Plasma ammonia	1 ml EDTA tube, on ice, to reach laboratory within 30 minutes	>600 µmol/l typical of UCDs 150 - 600 µmol/l typical of many OAs and Reye-like syndrome associated with FAODs
Plasma lactate	1 ml fluoride blood	Increase suspicion of OA where lactate and ketones are unable to explain the extent of an anion gap acidosis
Urine or plasma ketones	1 ml heparin blood on ice 1 ml plain urine	
Plasma laboratory glucose	1 ml fluoride blood	Confirm hypoglycaemia. Glucometers are unreliable during hypoglycaemia and can cross-react with galactose, giving erroneously elevated glucose levels in galactosaemia
Laboratory investigations Tier 2		
Urine organic acid analysis	2 ml urine in a plain tube, on ice	Confirms OAs. Detection of orotate in most UCDs. Increases suspicion for mitochondrial cytopathy
Plasma amino acid analysis	1 ml heparin blood, on ice	Confirms MSUD. Specific diagnosis in UCDs
Plasma acyl-carnitine analysis	1ml heparin blood, on ice	Confirms most OAs. Essential for confirmation of FAODs
Laboratory investigations Tier 3		
Plasma very long chain fatty acids	1 ml heparin blood, on ice	Essential for diagnosis of X-ALD, pyridoxine-responsive epilepsy and peroxisomal disorders (e.g. Zellweger's spectrum)
Plasma lactate/pyruvate ratio	1 ml fluoride blood on ice to reach laboratory immediately or blood collected in pre-prepared perchloric acid tube	A normal lactate/pyruvate ratio ≤20 obtained during lactic acidosis suggests pyruvate dehydrogenase deficiency or a gluconeogenic defect
Specific tissue or plasma enzyme assays	Plasma, leukocytes, erythrocytes, skin fibroblasts or hepatocytes collected as per testing laboratory protocol	Confirmation of specific enzyme deficiency
Molecular gene sequencing or mutational analysis	1 ml EDTA blood for nuclear gene mutations. Mitochondrial DNA preferably analysed in DNA extracted from affected tissue	Confirmation of genetic defect Family screening for carriers Antenatal diagnosis in future pregnancies

Table 3. Empirical management of suspected metabolic encephalopathy[6,13,14]

Intervention	Directions	Rationale
Halt protein and fat intake and replace calories with glucose	Dextrose IV at 10 mg/kg/min ± insulin to maintain normoglycaemia. If lactate >3 mmol/l and hyperglycaemia develops reduce glucose infusion rate and avoid insulin	Insulin and glucose promote anabolism and inhibit proteolysis/lipolysis that aggravate decompensation in OAs and UCDs. Glucose may stimulate hepatic glycolysis and lactate production
Manage hyperammonaemia aggressively	Ammonia >150 µmol/l: sodium-benzoate 250 mg/kg/day IV/O, and phenylbutyrate (450 mg/kg/day O) Ammonia >250 µmol/l: begin renal replacement therapy (dialysis)	Ammonia is a potent neurotoxin and can be effectively controlled with timely intervention
Correct metabolic acidosis	Ventilatory support, renal replacement therapy and $NaHCO_3$ infusion can be considered if plasma HCO_3- ≤15 mmol/l	Renal replacement therapy can significantly reduce toxic organic anion levels
Empirical administration of metabolic co-factors	Carnitine 100 mg/kg/day IV/O Hydroxycobalamin (vit B_{12}) 1 mg IM Biotin 10 mg/day O Thiamine 10 mg/kg/day IV Pyridoxine 10 mg/kg/day IV	Some disorders may respond dramatically to replacement of co-factors that have low toxicity and can be administered empirically pending a diagnosis

to life-threatening hyperammonaemic encephalopathy. Respiratory alkalosis is typically found due to stimulation of the respiratory centre by ammonia. Measuring plasma ammonia is critical for the early detection of UCDs and the prognosis is significantly improved if ammonia can be controlled quickly by protein restriction, renal replacement therapy and agents such as sodium benzoate and phenylbutyrate that can clear ammonia through alternate pathways. Liver transplant is curative, especially for severe defects. Milder defects are often managed with a combination of protein restriction, ammonia clearing agents and supplementation with arginine, an essential amino acid produced by the intact urea cycle. After demonstrating hyperammonaemia, a specific diagnosis can be made by analysing plasma amino acids, including citrulline and argininosuccinate, and by measuring orotate in urine. The diagnosis is confirmed by gene sequencing or tissue enzyme assay. Making a molecular diagnosis in X-linked recessive disorders such as OTC is particularly important as it allows for prenatal screening and the detection of female carriers in the family.

Mitochondrial cytopathies

The mitochondrial cytopathies represent a diverse group of IMDs that typically involve organ systems with high aerobic energy requirements such as muscle and nervous tissue. They are caused either by mutations in the maternally inherited mitochondrial genome (mtDNA), or by nuclear DNA mutations.[8] Due to the fact that mitochondria are not uniformly distributed and that mutated and normal mtDNA can occur in the same mitochondria (heteroplasmy), these disorders can present variably, even if the same mutation is involved. Some mitochondrial cytopathies may present with relatively acute-onset encephalopathy and lactic acidosis but this is usually preceded by progressive neurological deterioration with movement disorders, seizures, stroke-like episodes and neuropathy as examples.[10]

Mitochondrial cytopathies often also present with evidence of other progressive organ system dysfunction such as myopathy/cardiomyopathy, renal tubulopathy, hepatopathy, hearing and visual defects and endocrinopathies. Unfortunately a single effective screening test for this group of disorders does not exist.[9] Demonstration of lactataemia has poor specificity and sensitivity and the diagnosis is often made only after exclusion of other disorders. Working these patients up includes selective molecular screening for common mtDNA and nuclear DNA mutations, light and electron microscopy of affected tissues and specific mitochondrial enzyme respiratory complex enzyme assays performed on fresh tissue. Very few mitochondrial cytopathies are significantly amenable to treatment.

Laboratory work-up of suspected metabolic encephalopathy

Metabolic disease should be considered in any child presenting with progressive

or acute-onset neurological deterioration. Clinical suspicion must remain high where a reasonable alternative diagnosis is not immediately apparent, where the deterioration is associated with recognised catabolic stress, in neonates and infants presenting for the first time, or where a family history of similarly affected siblings is apparent.[11]

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A tentative diagnosis can often be made on clinical grounds together with the results of the Tier 1 investigations outlined in Table 2. Where clinical suspicion is high or where initial investigations support a metabolic diagnosis, Tier 2 investigations are appropriate. Tier 3 investigations are usually performed later to confirm a diagnosis or where clinical suspicion remains despite nonspecific results obtained at Tiers 1 and 2. The laboratory investigations listed will detect or infer a diagnosis in the majority of IMDs presenting with encephalopathy that may occur in South Africa, but clinicians need to remain cognizant of the fact that many other IMDs such as galactosaemia require directed diagnostic testing and may be missed by these screening tests.

Empirical management of metabolic encephalopathy

Emergency empirical management of a child with metabolic encephalopathy can significantly reduce morbidity, pending confirmation of the diagnosis by specialised investigations. The principles of empirical management are outlined in Table 3. These interventions are based on the control of life-threatening metabolic derangements, where benefits outweigh the potential risks. General supportive therapy should always be provided concomitantly. In patients where pyruvate dehydrogenase deficiency is strongly suspected, these guidelines should be adapted. These patients can present with encephalopathy and/or seizures, and may have subtle cephalic and facial dysmorphisms similar to fetal alcohol syndrome together with anatomical brain abnormalities and typically have very high cerebrospinal fluid and plasma lactate levels with normal plasma lactate/pyruvate ratios of ≤20.[12] They may respond to treatment with high fat and low carbohydrate intake. As soon as a specific metabolic diagnosis is available, interventions must be altered as per accepted guidelines.[13,14]

References available at www.cmej.org.za

In a nutshell

- Clinicians in South Africa need to rely on a high level of clinical awareness, combined with a low threshold for ordering basic metabolic investigations, to effectively diagnose and treat patients presenting with potential metabolic encephalopathy.
- Some treatable IMDs are relatively common in South Africa.
- The assumption that these disorders are collectively rare must be dispelled.
- As awareness increases, we hope that clinicians will increasingly utilise available laboratory and NBS resources, enabling more of these patients to receive the correct timely diagnosis and appropriate management.

Single suture

Hungry Africa's breadbasket needs to grow

Home-grown wheat could be the solution to a growing hunger problem in sub-Saharan Africa. The region is one of the few in which the number of undernourished people is rising, bucking a global trend. But a new analysis suggests wheat production there falls a long way short of what's possible.

A report from the UN Food and Agriculture Organization (FAO) concludes that the number of chronically undernourished people in the world has dropped in the last four years. Africa is the only region where the number has actually risen – by 20 million over the same period. The FAO says that agricultural growth there is essential.

Wheat could be the answer, say researchers at the International Maize and Wheat Improvement Center. At a recent conference in Addis Ababa, Ethiopia, they presented an analysis of 12 sub-Saharan countries. They conclude that in areas where conditions favour wheat growing, the yields are only hitting 10 - 25 per cent of their potential.

'[Extra wheat] would free locals from dependence on markets, where the price can rise by 50 per cent in a few months,' says Hans-Joachim Braun, head of the centre's global wheat programme. Braun says African ministers have contacted him, saying they want to grow wheat. The FAO report gives broad-brush guidance on where this might be feasible.

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