Morphological correlates of mitochondrial dysfunction in children

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Morphological studies have traditionally played a major role in the study of adults with suspected mitochondrial diseases. Here we review their role in the investigation of paediatric patients. The morphological changes may be macroscopic, such as developmental abnormalities of the brain in pyruvate dehydrogenase deficiency, including ectopic inferior olives and the absence of corpus callosum and pyramids. Other changes are histological, such as rarefaction of the neuropil and endothelial prominence in Leigh syndrome, and spongiosis with neuronal loss and gliosis in Alpers disease. The ragged-red fibres typical of mitochondrial disease in adults are only rarely seen in skeletal muscle biopsies from children. On the other hand, dramatic ultrastructural changes involving the mitochondria may be seen in many organs, including the liver, heart and intestine. In Alpers and lethal infantile mitochondrial diseases, the hepatocytes show marked accumulation of small droplets of lipid alternating with densely packed mitochondria with pale matrix and loss of granules. These changes are associated with a marked decrease in respiratory chain enzyme activity in the liver, often without similar decrease in the skeletal muscle or fibroblasts. Enlarged mitochondria with concentric cristae are prominent in the cardiac myocytes in Barth syndrome. For the assessment of children with a suspected disorder of mitochondrial dysfunction, detailed morphological studies of the brain (at autopsy) and of biopsies (especially of the liver), including ultrastructural assessment of the mitochondria, can be a very useful preliminary investigation. The findings should then be correlated with the clinical features and used as a guide for further biochemical and molecular studies, preferably on multiple tissues.

Key words: mitochondria/mitochondrial diseases/multiple tissues/pathology/ultrastructure

Introduction

The investigation of patients with primary mitochondrial dysfunction involves multiple disciplines: clinical medicine, neuroimaging (including computerized tomography, nuclear magnetic resonance and more recently positron emission tomography), pathology (in particular biochemistry), and molecular biology. Probably because the concept of mitochondrial diseases developed mainly from the early work of neurologists on patients with neuromuscular diseases, the role of anatomical pathology has
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for a long time been in the evaluation of skeletal muscle biopsies. The traditional hallmark of a mitochondrial disease is the ragged-red, or cytochrome oxidase-deficient, fibre. The ragged-red fibre is so named because of its appearance in a Gomori’s trichrome stain, where the dense collections of mitochondria appear brilliant red. Under electron microscopy, the mitochondria contain dramatic crystalline inclusions sometimes compared with parking lots. In many cities (including Melbourne), the study of skeletal muscle biopsies, with the extensive use of enzyme histochemistry, is performed by special units, and very few pathologists are exposed to this work. More recently the spectrum of mitochondrial diseases has expanded considerably. Physicians in many areas are becoming more aware of, and interested in, these conditions. Also many more different tissues are being biopsied. As a result many more pathologists are involved, although usually only on a very irregular basis and a multidisciplinary approach remains vital. In the face of the rapid development and change, the relevance of each discipline has to be assessed and modified regularly. We have reviewed the role of morphological studies based on our experience at a children’s hospital.

The material presented in this review is mainly from a systematic study of brains from autopsies over a 30 year period (1961–1990), and of biopsies subjected to electron microscopy over 15 years (1976–1990), to define the role of anatomical pathology in the investigation of patients with genetic diseases involving the central nervous system (Chow, 1996). For the present study, we have also included some more recent cases to expand the experience in a few of the more uncommon entities, as well as some cases with known mitochondrial dysfunction involving organs other than the brain. Most of the electron microscopy in this review was performed on non-muscular tissues. For many years, the muscle biopsies in the state of Victoria (Australia) have been assessed by a separate unit, although in the earlier years some of the material for electron microscopy was processed at the Royal Children’s Hospital, Melbourne. This material was also available for study. The cases presented here were those with a mitochondrial dysfunction, demonstrated either biochemically or at the molecular level. Only those with a clearly defined or probable diagnosis were included. Patients in whom the features were difficult to include under a specific diagnosis are not presented. At the present time, there does not appear to be a universally accepted classification of mitochondrial disorders. For the convenience of presentation, the classification proposed by Tyler (1992) is used. In this scheme the disorders are divided into: (i) defects of transport of substrates; (ii) defects of utilization of substrates; (iii) defects of the citric acid cycle; (iv) defects of the respiratory chain enzymes; and (v) defects of energy coupling. In this series there were no patients from categories (iii) and (v), both of which are exceptionally rare.

Abnormality of transport of substrate

Systemic carnitine deficiency

Carnitine is an amino acid derivative which facilitates the transport of fatty acids across the mitochondrial inner membrane. Patients with systemic carnitine deficiency may have a defect in carnitine transport, or the carnitine deficiency may be a secondary feature of various beta-oxidation disorders. The condition is usually manifested by muscle weakness, and occasionally heart failure and hepatic encephalopathy. A patient with systemic carnitine deficiency presented at five months after 2 weeks of vague ill health. Marked hepatomegaly and cardiomegaly were noted and a liver biopsy was performed. Histology showed uniform severe macrovesicular fatty change
involving the hepatocytes. Ultrastructure showed that not only did the hepatocytes contain large deposits of fat, but some sinus lining cells also contained prominent numbers of fat droplets. The mitochondria showed no conspicuous abnormality in number or structure. This patient died shortly afterwards. Specimens collected at autopsy showed that the carnitine concentrations in the heart, liver and skeletal muscle were 20, 15 and 20% of control values, thus confirming the diagnosis.

Abnormalities of utilization of substrate

Medium chain acyl-coenzyme A dehydrogenase (MCAD) deficiency

Medium chain acyl-coenzyme A esters are major substrates of mitochondrial metabolism. Patients with MCAD deficiency usually present with hypoketotic hypoglycaemia, precipitated by fasting. Occasionally the attack can be fatal and and the clinical history may resemble sudden infant death syndrome (SIDS). Morphology was only available in two patients, since in recent years the diagnosis has been based on mass spectrometry of the urine or molecular identification of the common A985G mutation, obviating the need for tissue study. One patient presented at 16 months with hypoglycaemia and severe hepatomegaly. Liver biopsy, including ultrastructure, showed marked fatty changes similar to the patient with systemic carnitine deficiency. Fatty change in the liver is an exceedingly common finding in pathology. However, the fat accumulation is usually confined to the hepatocytes. For the change to involve sinus lining cells is very rare. This feature should raise the possibility of a defect in the transport or utilization of substrates. The second patient presented with SIDS. Although in the state of Victoria, MCAD deficiency was routinely investigated in SIDS patients for many years by checking the common mutation, this was the only patient shown to have the condition. This experience is in agreement with several recent studies that suggest MCAD deficiency is not a common cause of SIDS (Holton et al., 1991; Arens et al., 1993). Both our patients were homozygous for the common A985G mutation.

Pyruvate dehydrogenase (PDH) deficiency

Pyruvate is another major substrate of mitochondrial metabolism. Most patients with PDH deficiency presented with abnormal neurological development and lactic acidosis. There was considerable variation in their clinical features. Some patients presented with Leigh syndrome (see below).

Two female patients presented in the neonatal period with intrauterine growth retardation, hypotonia, and dysmorphic features including adducted thumbs, single palmar creases, and pes cavus. The cerebrospinal fluid showed marked increase in lactate and pyruvate. They died at the age of 4 and 5 weeks. Autopsy of the brain showed a dramatic and identical combination of features in each, comprising marked dilatation of the ventricles, almost total absence of the corpus callosum, total absence of the pyramids and ectopic foci of the inferior olives. Although it clearly cannot be considered specific, this combination of developmental abnormalities in a neonate with primary lactic acidosis should prompt investigation of PDH deficiency (Chow et al., 1987; Michotte et al., 1993). Developmental abnormalities have traditionally not been associated with metabolic defects. However more examples are being described since the Zellweger or cerebro-hepato-renal syndrome was shown to be the result of a disorder of the biogenesis of peroxisomes (Goldfisher et al., 1973). An important relatively recent addition is the defect of cholesterol biosynthesis in the Smith–Lemli–Opitz syndrome (Irons et al., 1996).
Abnormalities of respiratory chain enzymes

Leigh syndrome

We have previously reviewed a series of 67 patients with Leigh or Leigh-like syndromes (Rahman et al., 1996), including 16 patients seen clinically and at autopsy at the Royal Children’s Hospital in Melbourne. The 67 patients were selected on the combination of clinical features, neuroimaging, lactate concentrations and neuropathology. The patients typically presented in early childhood with neurological regression, and brain stem signs such as irregular breathing, abnormal eye movements and ataxia. At autopsy the typical lesions were seen around the ventricular system particularly in the brain stem with marked rarefaction of the neuropil, vascular proliferation and often well-preserved neurons. Although neuropathology has traditionally been used as the benchmark for diagnosis, such a diagnosis is by itself insufficient for genetic counselling as the aetiology of Leigh syndrome is known to be diverse (Rahman et al., 1996), including abnormalities in the pyruvate dehydrogenase complex which are X-linked, point mutations of mtDNA which are maternally inherited and other nuclear encoded respiratory chain defects including mutations in the SURF-1 gene (Tiranti et al., 1998; Zhu et al., 1998). Skeletal muscle biopsies from four patients with mutations either in the SURF-1 gene or in mtDNA were available for study. In spite of clearly defined genetic defects, these specimens only showed occasional small clusters of lipid droplets mixed with mitochondria, with the latter showing no conspicuous abnormality of structure.

Alpers disease

Progressive neuronal degeneration of childhood with liver disease, i.e. Alpers disease, is a fatal disorder of childhood typically presenting with developmental delay, visual deterioration, explosive onset of intractable seizures and liver dysfunction. Six patients with Alpers disease were reviewed. In one patient the liver disease preceded the onset of neurological symptoms. Each of the patients showed typical neuropathology at autopsy comprising spongiosis of the cerebral cortex, particularly of the occipital lobes, and the deep nuclei, neuronal loss, and gliosis (Harding, 1990). The liver biopsies in all these patients showed a variable degree of microvesicular fatty change of the hepatocytes, although in two patients a first biopsy early in the course of the disease showed no abnormality. Electron microscopy showed alternating small droplets of fat and dense masses of mitochondria, some of which were markedly enlarged (Figure 1A). The mitochondria also showed pallor of the matrix, loss or fragmentation of granules, and compression of the cristae into stacks (Figure 1B). The consistency of the findings suggests that liver biopsy for electron microscopy and biochemistry can be a very useful diagnostic adjunct during life. In one patient the changes were seen when he presented with liver disease before the onset of neurological symptoms, thus excluding the possibility of liver cell damage due to Valproate, which is an important differential diagnosis in children treated with this drug for epilepsy. If Alpers disease is strongly suspected and the liver biopsy showed no abnormality, a repeat biopsy may be considered after an interval as sometimes the changes may not develop until later in the course of the disease. The ultrastructural changes have not always been noticed in other studies (Narkewicz et al., 1991), and the cause of this discrepancy is unclear. Perhaps it is related to the uneven distribution of the lesions or the timing of the liver biopsies.

Analysis of mtDNA did not show any large deletions or the common mutations. Biochemically there was a marked decrease
in the respiratory chain enzyme activity, particularly complex I, in the liver. The enzyme defect was absent or less obvious in the muscle and absent in the fibroblasts. Interestingly when there was a change in the morphology of the liver in a patient, the biochemical findings mirrored the change in histology (Table I).

**Lethal infantile mitochondrial disease (LIMD)**

Five patients were seen with a disease that was classified as LIMD. This term was chosen although the definition and minimum criteria of the entity are controversial. Most patients present with neuromuscular symptoms (Zheng et al., 1989; Bentlage et al., 1996), but there is considerable variation in phenotype. One patient may present with myopathy while a cousin has liver failure (Boustany et al., 1983). Also some infants presenting with cholestasis and progressive liver disease probably had the same condition (Ducluzeau et al., 1999). All our patients presented in infancy with hypotonia, developmental delay, abnormal liver function tests and marked primary lactic acidosis. They all died within or shortly after the first year. The liver showed extensive microvesicular fatty change and a variable degree of fibrosis. Ultrastructure showed alternation of small droplets of fat and densely packed masses of mitochondria, with pale matrix and indistinct granules (Figure 2A,B). Unlike those in Alpers disease, the focal marked enlargement and compression of the cristae were not seen. In one patient there was a history of a previous sibling who had died in infancy with liver failure. This patient was admitted with markedly abnormal liver function tests shortly after exposure to sucrose, and hereditary fructose intolerance was considered the most likely diagnosis. In spite of the dramatic changes in the mitochondria, it was felt important to check the aldolase activity, which was normal, thus excluding the diagnosis of hereditary fructose intolerance. Ultrastructural examination with systematic checking of different organelles should be part of the routine assessment of a liver biopsy when a metabolic disorder is suspected. This has been shown to be a very useful triage procedure, for selecting the most appropriate
Table I. Deterioration in liver respiratory chain enzyme activity in a patient with Alpers disease, accompanied by the development of morphological changes. Values are the residual activity of each complex as a percentage of normal mean activity standardized against citrate synthase activity (see Thorburn, 2000)

<table>
<thead>
<tr>
<th>Age at liver biopsy</th>
<th>Histology</th>
<th>Mitochondria</th>
<th>Complex I</th>
<th>Complex II</th>
<th>Complex III</th>
<th>Complex IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 years 4 months</td>
<td>normal</td>
<td>normal</td>
<td>79</td>
<td>90</td>
<td>72</td>
<td>82</td>
</tr>
<tr>
<td>6 years 3 months</td>
<td>focal microvesicular fatty change</td>
<td>abnormal</td>
<td>23</td>
<td>62</td>
<td>18</td>
<td>27</td>
</tr>
</tbody>
</table>

biochemical tests on a limited amount of liver tissue.

Analysis of mtDNA did not show any large deletions or any of the common mutations in these patients. Biochemistry showed marked reduction in the respiratory chain enzyme activity, particularly complexes I and IV. The defects were prominent in the liver but less obvious or absent in the muscle and fibroblasts, thus emphasizing the importance of examining multiple and appropriate tissues (Table II).

**Cardiomyopathy**

X-linked cardiomyopathy has been known for a long time, and some patients demonstrated dramatic changes in the mitochondria (Neustein, 1979). More recently the gene G4.5 which is responsible for the Barth syndrome, i.e. X-linked cardiomyopathy (usually dilated cardiomyopathy), skeletal myopathy, episodic neutropenia and short stature, has been cloned (Bione et al., 1996). The gene may also be responsible for other phenotypes involving the heart, such as isolated non-compaction of the left ventricular myocardium (Bleyl et al., 1997). Electron microscopy of the heart was performed in two patients. In the first patient, who had had a history of dilated cardiomyopathy for many years, endomyocardial biopsy was performed when he was 13 years old. Episodic neutropenia and an X-linked recessive family history were present although these were not noticed by the attending physicians. The diagnosis of X-linked cardiomyopathy was first suggested by the ultrastructure show-
Table II. Early alteration of liver respiratory chain enzyme activity in a patient with lethal infantile mitochondrial disease, demonstrating the much greater sensitivity of liver biopsy over muscle and fibroblasts in making the diagnosis of mitochondrial disease in children with liver dysfunction. Values are the residual activity of each complex as a percentage of normal mean activity standardized against citrate synthase activity (see Thorburn, 2000).

<table>
<thead>
<tr>
<th></th>
<th>Complex I</th>
<th>Complex II</th>
<th>Complex II + III</th>
<th>Complex IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblasts</td>
<td>75</td>
<td>76</td>
<td>126</td>
<td>64</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>32</td>
<td>128</td>
<td>92</td>
<td>118</td>
</tr>
<tr>
<td>Liver</td>
<td>0</td>
<td>50</td>
<td>14</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure 3. (A) Myenteric neuron in myo-neuro-gastrointestinal encephalopathy (MNGIE)-like syndrome with markedly enlarged mitochondria. Scale bar = 2.5 μm. (B) Enlarged myenteric neuronal mitochondria in MNGIE-like syndrome with pale matrix and loss of granules. Scale bar = 1.0 μm.

Kears–Sayre syndrome (KSS)

KSS was originally described in two patients with external ophthalmoplegia, retinitis pigmentosa and heart block (Kearns and Sayre, 1958). The clinical spectrum was subsequently expanded (DiMauro et al., 1985). The child...
we studied presented at 2 years with short stature and poor weight gain. Insulin-dependent diabetes was noted at 4 years. At 10 years, based on ptosis, progressive external ophthalmoplegia, and retinal pigmentary degeneration, KSS was diagnosed. A muscle biopsy showed ragged-red fibres, with prominent crystalline inclusions in the mitochondria on electron microscopy. A liver biopsy performed at the same time showed no conspicuous abnormality at light microscopy. At electron microscopy, the mitochondria showed moderate variation in size and shape with many containing paracrystalline inclusions. Such inclusions are however very common in the liver and can be seen in many conditions many of which are not primary metabolic disorders. The respiratory chain enzyme assays were not performed on the liver, but they were normal in the muscle, even though there were conspicuous abnormalities in the enzyme histochemistry and ultrastructure of that tissue.

**Pearson syndrome**

Pearson syndrome was described two decades ago as a rare condition with sideroblastic anaemia, vacuolated marrow cells and pancreatic insufficiency (Pearson et al., 1979). Great interest was raised when this was shown to be associated with deletion of the mtDNA 10 years later (Rotig et al., 1989). Since then the clinical spectrum of the condition has been widened. A patient may survive and later develop features of the KSS (Simonsz et al., 1992), or one member of the family may present with progressive ophthalmoplegia while another has Pearson syndrome (Bernes et al., 1993). Some patients may also present with severe renal tubular dysfunction (Niaudet et al., 1994), and these patients may show bizarre giant mitochondria in the kidney and muscle (Gilbert and Emms, 1996), suggesting that electron microscopy can be useful in the investigation of these patients. Only one patient was seen. He presented at 7 months with pallor and tiredness. Investigations showed a sideroblastic anaemia and vacuolation of myeloid and erythroid precursors in the marrow. He had systemic lactic acidosis and fat in the stools suggesting pancreatic insufficiency. Over the next 2 years he developed marrow aplasia, pneumonia and
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salt losing nephropathy, and died aged 3 years 4 months. Analysis of the mtDNA showed a 2.461 kb deletion (Kapsa et al., 1994). Only a needle core of the liver was obtained after death. The tissue was insufficiently well preserved for electron microscopy, but light microscopy showed focal fibrosis and marked haemosiderosis of the hepatocytes and Kupffer cells, similar to the findings in previous reports (Gurgey et al., 1996).

**Myo-neuro-gastrointestinal encephalopathy (MNGIE)-like syndrome**

Gastrointestinal symptoms have also been associated with mitochondrial dysfunction (Bardosi et al., 1987; Hirano et al., 1998). In the typical MNGIE syndrome, the patients present with ptosis, external ophthalmoplegia, gastrointestinal dysmotility, leukoencephaly, and polyneuropathy. A brother and a sister presented with severe intestinal dysmotility at the age of 12 and 8 years respectively. Various methods of feeding were tried unsuccessfully and total parenteral nutrition had to be given. They later developed peripheral neuropathy and proximal myopathy. The sister also developed myoclonic seizures and imaging showed focal areas of decreased density in both the cortex and subcortical areas. In the elder sibling a full thickness intestinal biopsy was performed when he first presented. Histology showed focal deficiency of the longitudinal fibres in the muscularis externa and prominent increase in lipofuscin granules in the smooth muscle fibres. Electron microscopy showed marked swelling of the mitochondria in some neurons in the myenteric plexus (Figure 3A,B). Although commented on by the observer, the significance of this feature was not appreciated until considerably later when the neurological symptoms became obvious. At that stage a liver biopsy was performed. The liver cells showed no significant fatty change. Many mitochondria contained paracrystalline inclusions. Biochemistry showed marked reductions in the activity of complexes I, III and IV, with increased complex II activity. Biochemistry of the muscle was normal. Analysis of the mtDNA showed no large deletions, nor any of the common mutations. These two siblings showed many features similar to MNGIE, but also some different ones, such as the relatively good nutritional status, lack of ophthalmoplegia and the lack of deletions of the mtDNA. However the severe decrease in respiratory chain enzymes and the markedly swollen mitochondria in the neurons of the myenteric plexus are strongly suggestive of a mitochondrial disorder. More recently mutations in the gene specifying thymidine phosphorylase, located on chromosome 22q13.32-qter, have also been shown to cause MNGIE (Hirano et al., 1998; Nishino et al., 1999).

The diseases and the associated morphological changes presented are summarized in Table III.

**Conclusions**

Morphological examination, including ultrastructural assessment of the number and structure of mitochondria can play a very useful part in the investigation of children with mitochondrial dysfunction. This investigation should remain a multidisciplinary approach, with each discipline complementing the others. In some diseases, a strong presumptive diagnosis may be made on typical pathological findings, in correlation with clinical features, but in most situations morphological studies should be considered a preliminary and triage procedure, to provide a guide on the decision for further biochemical and molecular investigations.

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References


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