Reye’s syndrome: diagnosis by muscle biopsy?

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SUMMARY Three children with Reye’s syndrome are described. One child died, the second had mild and transient illness, and the third had recurrent episodes. In all 3 children a muscle biopsy showed pronounced infiltration of the myofibres with fat microdroplets as shown by the oil red O stain and by electron microscopical examination. We suggest that needle biopsy of muscle may be a quick and safe aid to the diagnosis of Reye’s syndrome, and may be preferable to liver biopsy in view of the pronounced tendency to bleed in Reye’s syndrome.

Reye’s syndrome is known to be a cause of severe encephalopathy and liver failure in childhood.1 The prognosis used to be poor with a 70–80% mortality rate, but early diagnosis and treatment have improved the prognosis and reduced the mortality rate to 15–20%.2 It is important to distinguish Reye’s syndrome from other causes of hepatic failure as the treatment of Reye’s syndrome is different from that of the others.

In addition to the clinical and laboratory investigations that are routinely carried out,3 a firm diagnosis can be made on material biopsied from the liver by the finding of diffuse, microvesicular, fatty infiltration of hepatocytes without sign of hepatocellular necrosis or significant inflammatory infiltrate. However because of the risk of haemorrhage a liver biopsy should be postponed until the coagulation factors have been corrected. This may require techniques—such as exchange transfusion—which in themselves carry significant morbidity in older children. Percutaneous needle biopsy of muscle is less hazardous than that of the liver because there is less likelihood of severe bleeding.

It is the purpose of this paper to demonstrate that a muscle biopsy by needle is a useful, quick, and accurate aid to the diagnosis of Reye’s syndrome.

Case reports
Case 1. A 6-year-old Arab girl was admitted to this hospital with a history of vomiting and progressive loss of consciousness of 2 days’ duration. There was no history of pre-existing infection, headaches, or intoxication. The day before admission she had been treated at another hospital with intravenous glucose for hypoglycaemia and this had temporarily improved her level of consciousness. On admission, she was in semicoma and had sluggish responses to painful stimuli. Her blood pressure was 150/80 mmHg, pulse rate 80/min, respiratory rate 40/min. Pupils were equal and reacted to light, funduscopic examination showed no papilloedema. The oculocephalic reflex was normal. Muscle tone was generally decreased. Deep tendon and abdominal reflexes were decreased. Babinski’s sign was negative. The liver was palpable 4 cm below the costal margin. Treatment consisted of dexamethasone, arginine glutamate, ampicillin, vitamin K, mannitol, dopamine and, later, mechanical respiratory support. Her condition quickly deteriorated and she went into deep coma with Cheyne-Stokes respiration. The oculocephalic reflexes ceased and the pupils became dilated and no longer responded to light; she died in cardiorespiratory arrest. Necropsy was not performed but liver tissue was obtained by needle biopsy immediately after she died.

Laboratory findings
Hb 9.4 g/dl, haematocrit 34%, and platelets 240 × 10⁹/l, plasma urea 32.1 mg/ml (11.3 mmol/l), glucose 160 mg/100 ml (8.8 mmol/l), bilirubin 1.4 mg/100 ml (23.9 µmol/l), calcium 9.8 mg/100 ml (2.45 mmol/l), phosphate 5.0 mg/100 ml (1.6 mmol/l), uric acid 10 mg/100 ml (0.59 mmol/l), cholesterol 110 mg/100 ml (2.85 mmol/l), sodium 139 mmol/l, potassium 4.9 mmol/l, diastase 120 IU, aspartate transaminase (AST) 2000 IU, alkaline phosphatase 255 IU, prothrombin time 22%, partial thromboplastin time 16 seconds, arterial blood ammonia 532 µg/100 ml (312.3 µmol/l), total protein 5.6 g/100 ml (56 g/l), albumin 3.8 g/100 ml (38 g/l), Australia antigen negative.

The cerebrospinal fluid (CSF) was clear with no cells. Pressure was 300 mmH₂O, protein 29 mg/100
ml (0.29 g/l), glucose 65 μg/100 ml (3.6 mmol/l). Electroencephalogram (EEG) showed pronounced diffuse slowing which later became isoelectric.

**Special biochemical studies**

Plasma alanine 366 (normal <30) μmol/100 ml, plasma glutamine 392 (normal <50) μmol/100 ml, urinary lactic acid 325 (control 5–15) mg/100 ml, g lactic acid/g creatinine = 18 (control 0.24).

The activity of the liver ornithine-transcarbamylase was 757 μmol/g per hour (20% of normal activity). Enzymatic activity of the liver carbamylphosphatase-synthetase was 57 μmol/g per hour (half that of normal).

**Case 2.** A 7-year-old Jewish boy was admitted to the hospital because of persistent vomiting and progressive delirium for 12 hours. He had been in good health, except for a 3-day illness with pharyngitis one week before admission. There was no history of trauma or intoxication. On admission the boy was semiconscious, confused, and disoriented to time or place. He became delirious. Body temperature was 36.5°C, blood pressure 100/69 mmHg, respiration 24/min; cranial nerve examination, including funduscopy, was normal. The liver was palpated 7 cm below the costal margin and was tender, muscle tone and strength were diminished, but the tendon reflexes were brisk.

**Laboratory findings**

Serum glucose 86 mg/100 ml (4.8 mmol/l), bilirubin (total) 2.5 mg/100 ml (49.6 μmol/l), AST 425 units, blood ammonia (arterial) 163 μg/100 ml (95.7 μmol/l) (venous) 120 μg/100 ml (70.4 μmol/l), lactic acid 75 mg/100 ml (8.32 mmol/l), prothrombin time 45%, hepatitis antigen B negative. CSF pressure was 210 mmH2O, normal levels of glucose and chloride; no bacteria or virus isolated. The EEG showed marked generalised delta wave slowing. On admission the boy received an intravenous infusion with glucose 5% and, 2 hours later, dexamethasone. A great improvement in his condition was noted with this treatment. He regained full consciousness within 8 hours, and was fully recovered one week later.

**Case 3.** This 12-year-old boy was of Jewish-Ashkenazi extraction. At age 8½ years recurrent symptoms began and he had 6 acute episodes each one differing in severity. At onset he complained of headaches and vomiting and these had become progressively worse during the first day of illness and had required urgent admission to hospital. On admission he was found to be somnolent, apathetic, answering questions adequately but slowly. The neurological examination was normal except for brisk deep tendon reflexes. The skin looked yellow and the liver was palpable 25 cm below the costal margin.

**Laboratory findings**

Bilirubin (total) 2.5 mg/100 ml (42.7 μmol/l), AST 220 IU. CSF was found to be normal. The EEG showed generalised slowing. After this first episode 4 more attacks followed, 2 after marked exertion, one after an upper respiratory infection, and the last after a period of 24-hour fasting (day of atonement). In each episode the main symptoms were recurrent vomiting and varying degrees of coma. The liver was enlarged in each episode to between 3 and 5 cm below the costal margin. Review of the laboratory tests showed that bilirubin was increased up to 2.9 mg/100 ml (49.6 μmol/l), AST up to 2400 IU, glucose fell to 45 mg/100 ml (2.5 mmol/l) on one occasion. Blood ammonia was 140 μg/100 ml (82.2 μmol/l), free fatty acids 890 mmol/l. EEG showed generalised slowing during each episode.

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Fig. 1 (Case 1.) Electronmicrograph of hepatocytes with non-coalescing lipid droplets, and macrophage with numerous lysosomes of various sizes. (× 4000).
Histological studies

Case 1. Biopsies by needle from the liver and quadriceps muscle were available for study about 3 hours after admission. Cryostat section and glutaraldehyde-fixed material were prepared routinely for light and electron microscopical examination.3

Light microscopical examination of liver with Sudan black stain showed pronounced fatty infiltration in the parenchymal cells. In epon-embedded, toluidine blue stain 1 μm sections, it could be seen

Fig. 2. Electronmicrograph of swollen mitochondria inside hepatocytes. Note loss of cristae. (× 18000)

Fig. 3. (Case 1.) Cryostat section of muscle showing unremarkable changes in the lesion. (H and E × 100).

Fig. 4. (Case 1.) Same muscle stained with oil red O shows abundant fat droplets in most of the fibres. (× 100).

Fig. 5. (Case 1.) Myofibres containing lipid droplets (epon embedded 1 μm section toluidine blue stain × 300).
that the fat in each liver cell consisted of tiny droplets which did not coalesce. This was confirmed ultrastructurally (Fig. 1). The mitochondria were found to be extremely swollen and they had lost their cristae (Fig. 2). The muscle appeared normal as regards fibre size and type distribution (Fig. 3). There was a greatly increased amount of lipid especially in type 1 fibres, as demonstrated by oil red O (Fig. 4). Electron microscopical examination confirmed the excessive lipid (Figs 5 and 6).

In the interstitium, the endothelium of small blood vessels was swollen and contained numerous pinocytic vesicles.

Case 2. Muscle biopsy was performed from the quadriceps muscle. Light microscopical examination with haematoxylin and eosin stain showed no clear changes except for some differences in the size of fibre. On staining with NADH-TR and myosin ATP-ase the mosaic pattern remained, while the oil red O stain showed excessive lipid droplets in all myofibres, especially in those of type 1.

Electron microscopical examination of muscle confirmed the excessive lipid. There were also small foci of myofibrillar degeneration and glycogen accumulation.

Case 3. Muscle biopsy was performed by needle from the right quadriceps muscle. The biopsied material was freshly frozen, cryostat-sectioned, and glutaraldehyde-fixed.

Light microscopical examination with haematoxylin and eosin, NADH-TR, and myosin ATP-ase in pH 9-4 and 4-6 showed no changes, and a good mosaic pattern. Oil red O stain showed pronounced red staining in many of the fibres.

Electron microscopical examination showed a significant increase in the amount of fat droplets between the myofibres. Many mitochondria were dilated with changes in the cristae, and in some fibres a pronounced increase of small mitochondria could be seen close to the lipid droplets.

Liver biopsy with Sudan black stain showed marked fat droplet infiltration in the hepatocytes. Electron microscopical examination showed that each hepatocyte was loaded with small fat droplets.

Discussion

Any patient under age 20 years who develops acute neurological disturbance several days after a non-specific viral illness, and has recurrent vomiting and progressive disturbance in level of consciousness, should be suspected as suffering from Reye's syndrome.

Demonstration of hyperammoniaemia, raised serum transaminases, prolongation of the prothrombin time, and normal CSF at raised pressure suggest a diagnosis of Reye's syndrome.

Each of these 3 children had one form of Reye's syndrome. One had the fatal type, one the mild and transient, and the third had the recurrent form.4

Muscle biopsies in Reye's syndrome have been done before,6 and it was known that the disease process in Reye's syndrome affected the muscle as part of a widespread disorder. Serum creatine kinase, an indication of muscle membrane disruption, is known to be raised in most cases. Morphological abnormalities—such as an increased quantity of lipids in type 1 fibres, generalised intermyofibrillar oedema, mitochondrial disruption, and swelling of vesicular endothelium—have been demonstrated in serial muscle biopsies in 4 patients. The biopsied muscle in our patients showed similar changes. However, it is important to stress the strong positive
reaction with the oil red O stain in our patients. Three patients with hepatitis served as controls and their muscle showed a normal amount of lipids on the oil red O stain.

Muscle uses lipids as an alternative source of energy to carbohydrate, mainly by β-oxidation inside the mitochondrial. Small droplets of lipid can be present in normal muscle fibres, especially in type 1 fibres which have larger and more numerous mitochondria. Abnormal amounts of lipids accumulate in muscle in several disorders: in the lipid storage myopathies, either due to specific metabolic defect like carnitine deficiency, in several of the mitochondrial myopathies, and in corticosteroid myopathy.

One can speculate that the cause of the accumulation of lipids inside the hepatocytes and myofibres in our patients was a result of the mitochondrial damage and lack of ability to oxidise the lipids. Other evidence to support the suggestion that Reye's syndrome can be regarded as a specific mitochondrialopathy is given by the enzymatic studies of the urea cycle in the liver biopsy. In Case 1 low enzymatic activities of ornithine-transcarbamylase and carbamylphosphatase-synthetase were found; these are the only enzymes in the urea cycle which are intramitochondrial. The high levels of plasma lactate, alanine, and glutamine also suggest a defect in the intramitochondrial oxidation of pyruvate. Our results support the suggestion that the pathophysiological basis of Reye's syndrome is a primary generalised mitochondrialopathy.

Since muscle biopsy can be carried out when coagulation factors are too depleted to make liver biopsy safe, this technique should be investigated in a larger series of patients to confirm its value in the diagnosis of Reye's syndrome.

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