

CLINICAL STUDY

Haemolytic anaemia and acute liver failure – the initial manifestations of Wilson's disease

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Abstract: We describe a case of a 16-year-old girl with Wilson disease, which was initially presented as Coombs-negative haemolytic anaemia and acute liver failure. The diagnosis was based on the findings of low ceruloplasmin serum level and high copper levels both in serum and 24-hour urinary excretion. The patient underwent orthotopic liver transplantation. A DNA-based diagnostic tool confirmed Wilson's disease: the patient was p.H1069Q homozygote. Based on further molecular-genetic examinations in the family, Wilson disease was diagnosed seven days later in one of the patient's asymptomatic brothers. The proband's cousin was confirmed as a carrier of the p.H1069Q mutation (Fig. 1, Ref. 24). Full Text (Free, PDF) www.bmj.sk.

Key words: Wilson's disease, haemolytic anaemia, orthotopic liver transplantation.

Wilson's disease (WD; McKusick 277900) is an autosomal recessive hereditary disorder of copper metabolism appearing in 1:40 000 of live-born children (variation 1:18 000–700 000) (1). The cause of the disease resides in the deficiency of copper-transporting ATPase. The responsible gene ATP7B coding the transmembrane protein is primarily expressed in the liver, brain and placenta and localised in the 13q14.3-q21.1 region (2). To date, more than 300 mutations in the ATP7B gene have been described. The most frequently occurring mutation is the missense mutation p.H1069Q that develops in 37–63 % of the white population (3, 4, 5, 6, 7). In the Czech and Slovak population, this mutant allele develops in 57 %. The three other prevalent mutations c.3402delC, p.W779X and p.R778G range between 4 and 2 % (8, 9).

Hepatic, neurological and psychiatric manifestations of Wilson's disease occur in ca 20 % of patients. Reduced biliary excretion of copper causes accumulation of copper in tissues and organs; primarily in the liver and brain.

Based on case reports (10, 11, 12) it can be assumed that the full development of clinical picture of WD may be preceded by transient episodes of intravascular haemolysis. It is supposed that retained copper gradually accumulates in the cytoplasm of liver cells and after particular saturation, some of copper is released into lysosomes. In about 5% of the patients, this transfer is very

rapid and followed by necrosis of hepatocytes and fulminant hepatic failure. Part of intracellular copper is released into the bloodstream; it damages the erythrocytes, and acute haemolytic anaemia develops.

Case report

Family history: parents unrelated, mother – unspecified hyperbilirubinaemia, father – healthy, 2 brothers – healthy.

Clinical history: One year before the acute liver failure the patient demonstrated fatigue and joint ache, for 6 months she had been taking hormonal contraception because of disorders of the menstrual cycle. For two weeks her temperature had been almost 38 °C; cold, emesis, diarrhoea, followed by icteric skin and sclera. She was admitted to the University Hospital Brno.

On admission: 16-year-old female, 67 kg, 170 cm, blood pressure values 160/80 mmHg, pulse 95/min, afebrile, conscious, icteric skin and sclera, swelling of forearm, hands, lower extremities and genitals, heart action regular, intensity of systolic murmur in precordial 2/6, breathing natural with no secondary phenomena, abdomen on palpation sensitive around the navel, splenomegaly, hepatomegaly (liver and spleen +1 cm under the costal arch), intestinal peristalsis audible only feebly.

The blood count showed severe anaemia (Hgb 45 g/l, Hct 0.13 l/l, Ery $1.3 \times 10^{12}/l$, Plt $126 \times 10^9/l$), Coombs test was negative, Hgb A2 negative. Laboratory tests demonstrated signs of intravascular coagulation activation: Q INR 4.63, aPTR 2.09 sec, fibrinogen 1.6 g/l, antithrombin III 31.0 %, D-dimmer 2.73 ng/ml. *Biochemistry:* extreme hyperbilirubinemia (total bilirubin 803.7 $\mu\text{mol}/l$, conjugated 568.2 $\mu\text{mol}/l$), mildly increased aminotransferases (ALT 0.33 $\mu\text{kat}/l$, AST 2.11 $\mu\text{kat}/l$, GGT 1.58 $\mu\text{kat}/l$), reduced alkaline phosphatase in serum – ALP 0.11 $\mu\text{kat}/l$, ammonia from venous blood was normal, albumin 25.8 g/l, total

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Fig. 1. Liver biopsy of a 16-year-old female WD patient, homozygote p.H1069Q: Detection of copper with rubeanic acid: presence of minute black granules in hepatocytes, in some nodes only focally. Cirrhotic nodule on the opposite side of the figure is negative, only with bilirubin.

LD 5.93 $\mu\text{kat/l}$, urea 10.2 mmol/l, creatinine 109.1 $\mu\text{mol/l}$. Biochemical tests showed increased copper (Cu) in serum 33.3 $\mu\text{mol/l}$, low value of ceruloplasmin (CP) 0.16 g/l, increased 24-hour basal urinary Cu 87.1 μmol .

Other examinations: abdominal ultrasound: liver was not swollen and showed no focal changes, echogenicity and echostructures normal; curve from the central liver vein – a-wave missing, sign of reduced liver elasticity, gall bladder swollen. Abdominal CT: splenomegaly, lower density of liver, hydroptic gall bladder, haemodynamics of both kidneys disturbed, large ascites, relatively smaller pleural exudates. Eye examination: Kayser-Fleischer's (K-F) ring not proved. NMR of the brain – no anomalies typical of Wilson's disease detected.

WD was diagnosed on the basis of a low serum ceruloplasmin concentration, high total serum copper concentration and a 24-hour urinary copper excretion. Due to acute liver failure, heavy coagulopathy, presence of encephalopathy and haemolytic anaemia the patient underwent orthotopic liver transplantation. Liver biopsy: chronic active hepatitis intensively active in the stage of developing liver cirrhosis, strong presence of Mallory's hyaline, positive reaction to Cu (Fig. 1). Hepatic copper content was not carried out. The suspected diagnosis of WD was confirmed by molecular analysis of the ATP7B gene: homozygote p. H1069Q.

On the basis of further molecular-genetic examinations in the family WD was diagnosed seven days later in one of the patient's brothers. Brother: 11 years, no subjective disorders, liver not hypertrophied, neurological finding with no focal symptomatology, biochemical parameters before WD therapy: total bilirubin 9.6 $\mu\text{mol/l}$, ALT 5.05 $\mu\text{kat/l}$, AST 2.35 $\mu\text{kat/l}$, GGT 1.19 $\mu\text{kat/l}$, ALP 3.97 $\mu\text{kat/l}$, normal values of cupremia (Cu in serum 12.9 $\mu\text{mol/l}$) and ceruloplasmin (CP 0.21 g/l), increased 24-hour basal urinary Cu 4.37 μmol . After a D-penicillamine challenge test, 24-hour basal urinary Cu increased to 14.5 μmol . The

hepatic copper content was 1127 $\mu\text{g/g}$ of liver dry matter. Histological examination of hepatic tissue – hepatic fibrosis, heavy diffuse macro and micro vacuolar steatosis, bilirubin and Fe pigment negative, Mallory's toxic hyaline not detected, negative finding of copper with rubeanic acid. Abdominal ultrasound: liver 1–2 cm below the costal arch, no focal changes, echogenicity discretely higher, normal recording from central hepatic vein. Eye examination: K-F ring not confirmed. NMR of the brain: in FLAIR occipital symmetric hyper-signal foci up to 5 mm, in T2 possibly discrete non-homogeneity in thalami, discrete minute areas of hypo-density more to the left. DNA diagnosis of WD: homozygote p. H1069Q.

The proband's cousin was confirmed as carrier of the p.H1069Q mutation.

Discussion

Orthotopic liver transplantation (OLT) in WD patients is indicated in cases of fulminant form with acute liver failure that frequently occurs at the onset of the disease or after the interruption of therapy with chelating substances. Other indications are decompensated cirrhosis of the liver developing in spite of adequate chelating and supporting therapy, and advanced portal hypertension with recurring haemorrhage. Fulminant liver failure occurs more frequently in 18–22-year-old females. It has been speculated that it may be caused by estrogens (13).

In WD probands suffering from liver failure, the CP blood level is usually lower than 0.2 g/l. However, in the case of acute liver failure this marker is reduced regardless of its origin. Biochemical diagnosis of fulminant WD is usually based on a slight elevation in liver transaminases compared to the degree of hepatic insufficiency, elevated level of total bilirubin in blood, high Cu levels in 24-hour urinary excretion and the presence of Coombs-negative haemolytic anaemia (14). Although WD is suspected in cases of haemolytic anaemia and acute liver failure, it is not pathognomonic of the disease. A mild degree of haemolysis was detected in 50 % of patients with acute liver failure regardless of the cause. Likewise, an elevated 24-hour urinary excretion of Cu accompanies acute liver failure of different origin. The reason may reside in increased release of Cu from necrotic hepatocytes (15).

An important and specific symptom in WD patients with acute liver failure is a lower ALP level but its mechanism is not clear. It is assumed that hydroxyl or peroxide radicals induced by Fenton's reaction may reduce ALP activity by means of Mg^{2+} on ALP molecule (16).

In accordance with our findings, Eisenbach confirmed that a reduced level of blood ALT and haemoglobin could be a specific diagnostic marker in WD patients with acute liver failure. Other suitable markers are as follows: reduced AST activity, low cholinesterase activity, and high 24-hour urinary copper excretion. In patients with acute liver failure, the blood CP was reduced to less than 0.2 g/l regardless of its origin. Total blood bilirubin, ALP and albumin were insignificant for WD patients with acute liver failure (17).

Various scoring systems and mathematical methods are now used to assess the degree of liver failure using easily detectable pathophysiological values or clinical symptoms expressing the liver functions. They are designed for particular liver diseases or generally accepted, while not dependent on the aetiology of liver failure.

The simplest calculation is the value of ALP (UI/l)/total bilirubin (mg%) <2.0 or AST/ALT>4; in our patient these two markers were 0.14 and 6.39, respectively.

To distinguish fulminant WD (i.e. severe coagulopathy, INR>2, presence of encephalopathy or haemolytic anaemia) from decomposed form of WD (i.e. the presence of the known chronic liver disease with a sudden worsening of the situation with icterus and synthetic dysfunction of liver, with no encephalopathy or haemolytic anaemia) a number of prognostic indices can be used (17).

The so-called WD prognostic index (WPI) according to Nazer can be used to prognosticate the fatal result without liver transplantation: extension of INR, AST level and total bilirubin. A score of 6 or less indicates that survival is possible with chelation therapy; a WPI score of 7 and more indicates that without liver transplantation the patient will die (18).

In 2005 Dhawan revised this index and incorporated levels of blood albumin and the leucocyte count: the so-called RWPI (Revised WPI). A RWPI score of 11 and more is sufficiently sensitive, specific and has a negative predictive value above 90 % for death risk if liver transplantation is not done (19, 20, 21).

It has been reported that ca 80 % of patients survive one year after liver transplantation and 73 % survive 5 years. Ca 24 % of probands require re-transplantation of the liver (22). Within 6 months after liver transplantation, copper homeostasis should be settled and clinical symptoms of WD should subside. Our patient has survived 14 months and does not require re-transplantation of the liver.

The mechanism of the origin of haemolytic anaemia with WD has not yet been fully clarified. In WD patients, enormous amounts of copper are released from the liver into the bloodstream during haemolysis. In such a case, the level of free copper is comparable to copper poisoning. Free radicals, produced by the effect of free copper, are thought to be the cause of acute haemolysis in WD patients. Other potential mechanisms are the inhibition of erythrocytary enzymes and impairment of the erythrocytary membrane, which reduces ATP utilisation.

Attri explored the metabolism of erythrocytes and oxidative stress as the potential mechanism of haemolysis in 8 WD patients with haemolytic anaemia, in 8 WD patients without haemolysis treated with penicillamine, and in 8 healthy individuals. Elevated levels of free copper were detected in all WD patients with haemolysis. In this group, we saw inhibition of the erythrocytary enzymes: hexokinase, ATPase and glucose-6-phosphate dehydrogenase as compared with WD patients without haemolysis and healthy individuals. These findings confirm the hypothesis of impaired metabolism of erythrocytes during haemolysis in cases of WD. Furthermore, in this group we saw lower levels of anti-oxidative enzymes: super oxididismutase, catalase, glutathione peroxidase and glutathione reductase. Lipid peroxi-

dation increased and the levels of plasma antioxidants – uric and ascorbic acid – decreased. The authors assume that the production of free radicals by means of free copper, which changes the catalysed reactions, disturbs the oxidation. In WD patients with haemolysis the erythrocyte metabolism is altered and antioxidants are severely impaired (23).

Recent studies have shown that the theory of the production of free radicals and oxidation disorder is rather universal because this effect is too general and affects lipids, proteins, nucleic acids, mitochondria, cellular membranes etc. WD appears to be caused by the accumulation of copper in cells undergoing cirrhotic changes but only occasionally causes haemolytic anaemia. It was discovered that Cu (2+) causes cellular apoptosis by activating acid sphingomyelinase and releasing ceramides. The genetic deficit or pharmacological inhibition of acid sphingomyelinase protects against copper-induced cellular apoptosis of a transgenic mouse for WD. The activity of acid plasma sphingomyelinase was discovered to be higher and the level of ceramides and phosphatidylserine on the erythrocytic surface was increased in WD patients as compared to healthy individuals. It is assumed that this could be the mechanism at a molecular level that causes cirrhosis and haemolytic anaemia in WD patients. Whether the same mechanism also works in the brain is not yet known (24).

Conclusion

Diagnosis of Wilson's disease is very complicated. The variety of clinical symptoms may cause that the first symptoms of the disease are not diagnosed and therapy started as late as after a long interval of time. At the present time the accepted standard therapy for patients with hepatic forms of WD is therapy with zinc and chelates. In the final stage the only solution is liver transplantation.

Molecular genetic analysis can considerably help to assess WD diagnosis when the clinical expression is not quite unambiguous in order to ensure a timely diagnosis and treatment in so far undetected cases.

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