Screening for Wilson's disease in patients with liver diseases by serum ceruloplasmin

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Background/Aims: A low serum ceruloplasmin level is considered a diagnostic test for Wilson's disease. To examine whether it is useful to detect presymptomatic patients with Wilson's disease, serum ceruloplasmin was determined by radial immunodiffusion (normal: 20–60 mg/dl) in all patients (n=2867) admitted for evaluation of a liver disease in 1993 and 1994.

Methods: Patients with levels lower than 20 mg/dl were further evaluated by determination of serum copper concentration, urine copper excretion and ophthalmological examination. If possible, a liver biopsy was performed and the hepatic copper content was determined by flame atomic absorption spectroscopy.

Results: Seventeen patients had serum ceruloplasmin levels <20 mg/dl. One had asymptomatic Wilson's disease (no Kayser-Fleischer rings or neurological symptoms). In the other 16 patients Wilson's disease was excluded. Based on elevated hepatic copper concentration, three were considered as heterozygous carriers of the WD gene. The remaining patients had various liver diseases (acute viral hepatitis in three, chronic hepatitis in two, drug-induced liver disease in three, alcoholic induced liver disease in two) or malabsorption (n=3).

Conclusions: The positive predictive value of low serum ceruloplasmin was only 5.9%. Although helpful for identifying presymptomatic Wilson's disease, screening by determination of serum ceruloplasmin in unselected patients with clinical or laboratory evidence of liver disease is neither feasible nor cost effective.

Key words: Ceruloplasmin; Serum copper concentration; Wilson's disease.

Ceruloplasmin is an alpha 2-glycoprotein which is mainly synthesised by the liver (1). Its precise physiological functions are unknown; and may be related to copper transport (2), iron metabolism (3) and antioxidant defence (4). Ceruloplasmin certainly plays a role in the acute phase reaction. Its serum level is increased during inflammation and/or tissue damage (5) and in various malignancies (6).

Serum ceruloplasmin is considered a useful laboratory test for Wilson's disease. Its serum concentration is decreased in up to 95% of symptomatic patients (7). However, ceruloplasmin may be decreased in other disease states like fulminant hepatitis (8), decompensated cirrhosis (9) and protein wasting states (10). Furthermore, ceruloplasmin levels may also be low in patients with hereditary hypoceruloplasminemia (11).

Wilson's disease is an inborn error of copper metabolism that results in abnormal copper deposition in several organs such as the brain, liver and cornea (12). The most common clinical symptoms of Wilson's disease are symptomatic liver diseases, and/or typical extrapyramidal dysfunctions. Most patients with symptomatic Wilson's disease also have detectable Kayser-Fleischer rings. But also Kayser-Fleischer rings often are lacking in the absence of neurological symptoms and they certainly cannot be expected to occur in all cases of hepatic Wilson's disease. In the presymptomatic stage patients may show laboratory evidence of mild liver disease (i.e. increased ALI/AST) but diagnosis may become quite difficult (13). Serum alkaline phosphatase activity is frequently low in severe wilsonian damage (14,15), but is normal or
Ceruloplasmin as screening parameter

even increased in patients with mild liver disease (16). A ratio of total serum bilirubin concentration and alkaline phosphatase activity may differentiate fulminant Wilson's disease from other forms of fulminant hepatic failure. However, the usefulness of this test was not confirmed in larger series (17). If diagnosis can be made in the presymptomatic stage, appropriate treatment can prevent progression to more advanced and debilitating disease. One possibility for early diagnosis is to include measurement of serum ceruloplasmin as a screening test in the evaluation of patients with liver disease. The usefulness of this approach has not been studied so far. Therefore in this study ceruloplasmin levels were measured in all patients referred for suspected liver disease of any aetiology over a 2-year period.

Patients
Over a period of 24 months, ceruloplasmin levels were determined in 2867 patients referred for evaluation of liver disease.

Patients with serum ceruloplasmin levels lower than 20 mg/dl were further evaluated by slit lamp examination, by determination of serum copper concentration and 24-h urine copper excretion, and by testing for the most common mutation (His1069Gln) in Wilson's disease. If possible, a liver biopsy was performed.

Materials and Methods

Liver biopsy
For light microscopic examination sections were stained with H&E, Masson-Trichrome, Rhodamine, and Prussian Blue. Liver biopsies were evaluated using both a conventional descriptive diagnosis (CPH, CAH, cirrhosis, steatosis, fibrosis) and the classification of Scheuer (18).

Parts of the liver biopsy were dried and the copper content was determined by flame atomic absorption spectroscopy according to Kingston & Jassie (19) (normal: below 50 g/g dry weight; diagnostic range for Wilson's disease: >250).

Laboratory measurements
Ceruloplasmin in serum was measured by radial immunodiffusion (Nor Partigen®, Coeruloplasmin, Behring, Marburg, Germany; normal: 20–60 mg/dl). This reference value was obtained in 1453 healthy adult Central Europeans (773 males and 680 females) (20). Copper concentration in serum (normal: 65–165 g/dl) or in urine (normal: up to 100 g/d) was determined by flame atomic absorption spectroscopy. The collection of the 24-h urine copper excretion was done with great accuracy, using copper-free containers.

PCR-test for the His1069Gln mutation
High-molecular-weight DNA was isolated from whole peripheral blood or lysed mononuclear cells according to standard procedures. Exon 14 of the Wilson's disease gene was amplified by PCR from samples of 100–200 ng genomic DNA using the two intronic primers 3348 and 3349 as described previously (21). Briefly, amplification was performed in a Perkin-Elmer 2400 thermocycler using 33 cycles, each consisting of 20 s denaturation at 94°C, 30 s of annealing at 58°C and 25 s extension at 72°C. The resulting 337 base pairs (bp) product was then used as the template for a second PCR using the mismatch primer MUT 1069=5'TGCCGAGGAGCCAGTGACG3' (mismatch underlined) and the complementary intronic primer 3348 from the exon amplification. The product of this reaction was digested for 2 h with BsuHKA1 (New England Biolabs, MA, USA). The digested samples were then electrophoresed through a 9% nondenaturing polyacrylamide gel and stained with ethidium bromide. DNA fragments were photographed under UV transillumination.

Results
During the 2-year study period among 2867 adults with various liver diseases, 17 (0.59%) with serum ceruloplasmin levels <20 mg/dl were identified. Seven of them were referred for evaluation of various uncharacteristic liver diseases (elevated total serum bilirubin in two, elevated ALT in five). None of them showed evidence of viral, autoimmune, or toxin-induced liver diseases. Ten had well defined symptomatic liver disease (three acute viral hepatitis, two chronic viral hepatitis, two decompensated alcoholic cirrhosis and three drug-induced acute liver disease). Table 1 lists the clinical data from all these 17 patients.

One patient was diagnosed as having Wilson's disease. Patient L.D. consulted her physician because of severe metrorrhagia. Otherwise she was asymptomatic. Routine laboratory testing revealed mildly increased alkaline phosphate levels. Urinary copper excretion and the hepatic copper content were markedly increased. She was a compound heterozygote for the His1069Gln mutation. No Kayser-Fleischer rings were found at slit lamp examination. Brain NMR, recording of evoked EEG responses and routine clinical neurological examination were normal. Later on, her younger sister was also diagnosed as having Wilson's disease.

Based on elevated (>50 µg/g) hepatic copper concentration, three patients were considered to be heterozygous carriers of the Wilson's disease gene. All three patients were negative for the His1069Gln mutation.
Liver biopsy revealed stage II hepatic fibrosis in patient P.L., steatosis with rhodamine-positive granules in patient A.S., and a normal liver in patient R.J. R.J. was referred for evaluation of unconjugated hyperbilirubinemia (total bilirubin: 5-7 mg/dl) and fatigue. Examination revealed a Coombs-negative hemolytic anemia. All other possible reasons for hemolysis were excluded. At repeated examinations, serum ceruloplasmin level ranged between 10 and 16 mg/dl. Free serum copper was between 9 and 23 μg/dl. Kayser-Fleischer rings were not detected. The radio-copper test was abnormal (24 h/2 h copper ratio: 0.58; normal range >0.5, heterozygous range >0.5). On 125 mg zinc sulfate TID, serum-free copper decreased to zero and total bilirubin to<3 mg/dl.

In the remaining 13 patients with low ceruloplasmin levels, Wilson's disease was excluded with a high degree of certainty. Ten patients had known liver diseases of various origin including one patient with chronic hepatitis C (A.M.) with elevated hepatic copper content. One had celiac disease (K.O.) and his serum ceruloplasmin levels became normal on a gluten-free diet. No final diagnosis was made in two patients (W.E., M.E.).

Discussion
In the absence of typical clinical symptoms, the diagnosis of Wilson's disease is difficult (13). Mild abnormalities of liver enzymes may precede clinically overt disease. Therefore “screening” such patients by measuring serum ceruloplasmin levels may help to identify patients with Wilson's disease. In this study one patient with Wilson's disease and subsequently her younger sister were identified by this approach. However, the results of this study indicate that routine determination of serum ceruloplasmin in unselected patients with clinical or laboratory evidence of liver disease is neither feasible nor cost effective to diagnose presymptomatic Wilson's disease. Sixteen of the 17 patients with decreased ceruloplasmin levels did not have Wilson's disease.

Although in this prospective study 2867 newly referred adult patients with liver disease were tested for their ceruloplasmin values, calculation of the sensitivity and specificity of ceruloplasmin to diagnose Wilson's disease was not possible. First, patients with known Wilson's disease or potential heterozygotes referred for family screening were excluded. Second, the substantial number of patients examined may still not be sufficiently large in a disease with a prevalence of 1:30 000. This value is calculated based on clinical symptoms and/or conventional biochemical parameters (22,23). However, the prevalence of Wilson's disease among patients with liver diseases may be considerably higher, which is reflected by the finding of one asymptomatic patient among 2867 in this study. Based on a gene frequency of 1:90 (13) one would expect to find at least 30 heterozygotes. Since 80-90% of heterozygotes have normal ceruloplasmin levels (24) the three heterozygotes (P.L., R.J., A.S.) identified in this study by low ceruloplasmin levels are close to the
expected figure. Finally, it is possible that the diagnosis of Wilson's disease was missed in some patients because of normal ceruloplasmin levels. In the previous study, 35% of patients with hepatic Wilson's disease had normal serum ceruloplasmin levels and no Kayser-Fleischer rings (25). This can be explained by the increase of ceruloplasmin levels in response to pregnancy, oestrogen therapy, or acute inflammation (26,27) or by the overestimation of serum ceruloplasmin levels. The low positive predictive value (5.9%) of serum ceruloplasmin reflects the possibility that it is also low in non wilsonian patients by the immunological ceruloplasmin assay used in this study.

Apoceruloplasmin may interfere with this assay. Its contribution to the results obtained in patients with low ceruloplasmin values is negligible, but in those closer to the borderline, the additional apoceruloplasmin may push them into the normal range (28,29).

An overestimation of serum ceruloplasmin can be suspected if the serum copper concentration is lower than expected by the measured ceruloplasmin level (which contains 0.3% copper).

Such problems can be avoided by the oxidase method for ceruloplasmin determination, but this method is not generally available.

The serum ceruloplasmin levels may also be decreased in patients with fulminant hepatic failure of any aetiology (30), in about 25% of children with CAH (8), normal neonates (31) and patients with intestinal malabsorption, nephrosis, or malnutrition (32). In patients presenting with liver disease of known aetiology, the diagnosis of Wilson's disease may become a dilemma. Very low levels were found in a patient with autoimmune hepatitis which increased following steroid treatment (33). In patients with chronic hepatitis C, borderline ceruloplasmin levels are observed. In this study one patient (A.M.) with chronic hepatitis C had low serum ceruloplasmin and an increased hepatic copper content. However, the diagnostic value of hepatic copper determination in patients with chronic liver disease is unknown, since chronic liver disease per se may be associated with increased hepatic copper levels.

In summary, routine determination of serum ceruloplasmin concentrations in patients with liver disease may help to identify cases of Wilson's disease. However, the yield may be increased if only patients with liver diseases of unknown origin are screened and combined with the measurement of the 24-h urinary copper excretion.

Screening for well defined mutations of the Wilson's disease gene may help to identify asymptomatic patients (21). However, in view of the more than 60 mutations of the Wilson's disease gene described so far (34) it seems unlikely that such screening will be available in the foreseeable future.

References

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