Pilot study of screening for Wilson disease using dried blood spots obtained from children seen at outpatient clinics

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Summary: Wilson disease (WD) is an autosomal recessive disorder of copper accumulation leading to liver and/or brain damage. In this paper, we describe the results of a pilot study of screening for WD using ceruloplasmin determinations in dried blood samples. Specimens were collected from children aged 1 to 6 years who were seen at local paediatric outpatient clinics in the Miyagi Prefecture. We measured ceruloplasmin (CP) concentrations in 2789 children using an enzyme-linked immunosorbent assay. The mean value was 12.4 ± 3.95 mg/dl blood. Among these children, we identified two (case 1, male, 2 years old; case 2, female, 3 years old) with markedly reduced CP concentrations. Apart from low serum copper concentrations, their biochemical findings were almost normal, as were growth and development. To confirm the diagnosis, we analysed the WD gene and detected A803T/2871delC mutations in case 1 and R778L/G1035V mutations in case 2. We conclude that these children were presymptomatic WD patients. The CP level in dried blood samples from children aged 1 to 6 years appears to be a reliable marker for early detection of WD.

Wilson disease (WD; McKusick 277900) is an autosomal recessive disorder of copper accumulation, characterized by reduced incorporation of copper into ceruloplasmin (CP) and by decreased biliary copper excretion (Danks 1995). The disease is progressive and ultimately fatal if untreated, and is probably the most frequent cause of chronic liver disease in children. The estimated incidence of the disease is
approximately one case in 30,000–40,000 in Japan (T. Aoki et al, unpublished data), which is higher than that of phenylketonuria.

It is estimated that at least half of the patients with WD are never diagnosed and die of untreated disease (Brewer and Yuzbasiyan-Gurkan 1989). This occurs primarily because the disease is rare, and patients may have rather non-specific clinical presentations. This is unfortunate because the disease can be treated effectively once the diagnosis is made. Early diagnosis is needed to prevent the progression of liver injury or the onset of disabling neurological complications. The CP concentration is reduced in 95% of WD patients and has been considered a good marker for the detection of WD (Aoki and Nakanishi 1977; Endo et al 1994; Saito 1981). In this study, we performed preliminary screening for WD using CP determinations in dried blood samples collected from children aged 1 to 6 years.

MATERIALS AND METHODS

Blood samples were obtained on filter paper (routinely used for neonatal metabolic screening) by venepuncture in children aged 1 to 6 years who were referred to paediatric outpatient clinics for minor illnesses. Blood was drawn from these children during the physical examination and one drop from each child was used for WD screening. Sixty-five paediatric clinics in Miyagi Prefecture collaborated with us in the collection of samples. Samples were dried at room temperature, mailed to our laboratory within one week, and stored at 4°C until the assay was performed. Before sampling, informed consent was obtained from the parents of all children.

For the CP assay, a 3 mm disc was manually punched from each specimen. CP was eluted with phosphate-buffered saline. The Ceplatone.W kit (Nissho Co., Kusatsu, Japan) was used to determine the amount of CP in the eluate (100 μl). The enzyme immunoassay plate was pre-coated with ID1 antibody, and peroxidase-labelled ID2 antibody was used as the second antibody (Hiyamuta and Ito 1994; Hiyamuta et al 1993). The colorimetric assay was performed according to the manufacturer’s instructions. CP levels below the 3rd centile were arbitrarily considered abnormal and were confirmed by retesting the sample. When a low CP concentration was confirmed, a repeat dried blood sample was requested from that child. Serum total and active ceruloplasmin concentrations were assayed as described previously (Hiyamuta and Ito 1994; Hiyamuta et al 1993).

For 21 exons of the WD gene (ATP7B gene), amplification was performed with the sets of intronic primers described elsewhere (Petrukhin et al 1994; Thomas et al 1995). PCR was carried out in a GeneAmp 9600 (Perkin Elmer Cetus) with Taq polymerase (Pharmacia Biotech). PCR products were purified using a SUPREC-02 (Takara, Japan) and then directly sequenced in an automatic DNA sequencer (Hitachi, SG5500) with the Thermo-Sequenase cycle-sequencing kit (Amersham) (Yamaguchi et al 1997).

RESULTS

CP concentrations in dried blood spots: Ceruloplasmin concentrations were measured in samples from 2789 children from August to December 1996. The