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ORIGINAL ARTICLE

Clinical Features and Therapeutic Response in Taiwanese Children With Wilson's Disease: 12 Years of Experience in a Single Center

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Background: Wilson's disease (WD) is an autosomal recessive defect of cellular copper export. Early diagnosis in children is difficult due to its obscure clinical presentations. The efficacy of zinc salts is well documented, although there are limited data concerning zinc use in pediatric patients with WD.
Methods: We performed a retrospective analysis of clinical features, laboratory results and treatment responses in children with WD diagnosed at Taichung Veterans General Hospital between 1996 and 2008. Diagnosis was established by low serum ceruloplasmin, high 24-hour urinary copper excretion, presence of Kayser-Fleischer rings, and mutation analysis.
Results: Eleven children were included in this study. The main initial presentations were impaired liver function tests (6/11) and hemolytic anemia (2/11). Gene studies in seven children showed six different mutations (G934D, R778Q, C490X, 304insC, IVS4-1 G > C, P992I) and one possible novel mutation (L1181P). All patients had improved liver function tests and hemoglobin levels after treatment with D-penicillamine, trientine and zinc supplement therapy. During a mean period of 3.4±2.1 years with zinc therapy, six patients had serum zinc levels above the normal limit, and seven patients had serum copper levels below the normal range.
Conclusion: Serum ceruloplasmin and 24-hour urinary copper examinations could be used to rule out WD in children with chronic hepatitis and hemolytic anemia. Gene analysis is helpful for prompt diagnosis of asymptomatic siblings and patients with atypical features. Zinc treatment is generally safe in pediatric patients with WD. However, its adverse effects should be monitored.

1. Introduction

Wilson's disease (WD) is an autosomal recessive defect of cellular copper export related to the loss of functional ATP7B protein.[1,2] The worldwide prevalence is 1 in 35,000–100,000 live births.[3] The two most fundamental disturbances of copper metabolism in patients with this disorder are reductions in the rate of incorporation into ceruloplasmin and in biliary excretion of copper, leading to copper

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accumulation.[4] The clinical manifestations result from copper accumulation in various organs, with the most common presentations being hepatic or neuropsychiatric.[5,6] The signs and symptoms caused by copper deposition develop over a period of time, and WD is therefore difficult to diagnose in pediatric patients because of its obscure clinical presentations during the early stage of this disorder.[7] In children, it usually presents after 3 years of age with either incidental findings of abnormal liver function or as chronic disease, and rarely as hemolytic anemia and acute hepatic failure.[6] According to the guidelines of the American Association for the Study of Liver Disease (AASLD), genetic analysis is valuable for the diagnosis of asymptomatic siblings and patients presenting with atypical features of WD.[8] Although there have been several reports on children with WD in Asia,[9,10] studies of genetic diagnosis of WD are rare. Early diagnosis of WD is crucial because the disease may be fatal if left untreated.[1,11] Although zinc therapy is effective for children with WD, it may cause copper deficiency and excess of serum zinc.[12] In this study, we investigated the clinical features, diagnosis, genetic analysis and therapeutic outcome in children with WD.

2. Materials and Methods

2.1. Study population and diagnosis

We performed a retrospective analysis of the clinical presentations, laboratory findings, diagnosis, and therapeutic outcomes of pediatric patients with WD diagnosed at Taichung Veterans General Hospital between 1996 and 2008. The diagnosis was based on a combination of clinical, biochemical and genetic evaluations. These included low serum ceruloplasmin, high 24-hour urinary copper excretion, presence of Kayser-Fleischer rings (K-F rings), histologic signs of chronic hepatitis, and detection of gene mutations. With regard to genetic analysis for confirmation of diagnosis, the most common Taiwanese mutation positions for WD, including exons 2, 3, 5, 8, 12, 13, 16 and 18 of ATP7B, were screened by direct DNA sequencing.[4,13] The new Wilson index scoring system was used to evaluate the severity of the disease in our patients. Patients with a new Wilson index score of 11 or above were recommended to receive a liver transplant.[6]

2.2. Treatment and follow-up

All patients in this study initially received D-penicillamine (D-PCN) or trientine therapy, with or without zinc. Maintenance therapy with zinc acetate alone began if there were no signs of hepatic decompensation, and was administered at a dosage of elemental zinc acetate of 25 mg twice a day, until the age of 6 years, 25 mg three times a day between the ages of 7 and 16 years or until the child attained a body weight of 56.7 kg (125 pounds), and 50 mg three times a day thereafter.[12,14] We analyzed serum levels of copper and zinc, blood cell counts, and liver function tests during therapy.

3. Results

3.1. Clinical presentations and laboratory data

Eleven children, including nine boys and two girls, with a mean age of 10.0 ± 5.3 years (range, 2.5–17.7 years) were included in this study. Three of them were under 5 years old at diagnosis. The interval from symptom onset to diagnosis was 8.8 ± 1.3 months. The initial presentations were: impaired liver function tests (6/11), hemolytic anemia (2/11), fulminant hepatic failure (1/11) and liver cirrhosis (1/11). Case 3 was diagnosed because his brother had WD. None of them had neurologic symptoms or signs at presentation. Low serum ceruloplasmin and high 24-hour urinary copper excretion were noted in all patients except one, whose ceruloplasmin was not checked. Levels of urinary copper exceeded 100 μg/24 hours in all patients. Nine patients underwent liver biopsies, and the most common pathologic findings were chronic hepatitis (7/9) and steatosis (5/9; Table 1).

3.2. Diagnosis

Genotyping was performed in seven patients (cases 2, 3, 5, 6, 7, 8 and 9), and all patients showed mutations in both alleles. Of the seven different mutations identified, six known mutations were detected: G934D, R778Q, C490X, 304insC, IVS4-1G>C, P992I, as well as one possible novel mutation L1181P (Table 2). Cases 4 and 11 were diagnosed on the basis of the combination of low serum ceruloplasmin, high 24-hour urinary copper, and the presence of K-F rings, meeting the criteria of the AASLD's guidelines. Although case 1 only had data for low serum ceruloplasmin and high 24-hour urinary copper, his liver function parameters improved to a stable condition after D-PCN and zinc therapy during an 11-year follow-up period. The clinical course was thus compatible with a diagnosis of WD. Case 10 had 24-hour urinary copper levels well in excess of 1600 μg/24 hours. This finding excluded the possibility of autoimmune hepatitis, primary sclerosing cholangitis, and other causes of acute liver failure,[8] which are associated with 24-hour urinary copper levels below 1600 μg/24 hours. An alkaline phosphatase/total bilirubin ratio of less than 2.0 also

Table 1 Clinical features and laboratory examinations in the 11 patients with Wilson's disease*

No.	Sex	Initial presentation	Time from S/S to Dx (mo)	Serum ceruloplasmin (g/L)	Urine copper (μg/24h)	Pathology	Gene mutation
1	M	Impaired LFT	4	Undetectable	775.0	CH,S	ND
2	M	Hemolytic anemia	53	Undetectable	462.0	CH,S	G934D/R778Q
3	M	Family history: +	1	Undetectable	294.0	CH,S	G934D/R778Q
4	F	Hemolytic anemia	1	Undetectable	3075.0	CH,LC	ND
5	M	Impaired LFT	5	Undetectable	112.0	CH	304insC/IVS4-1 G→C
6	F	Impaired LFT	8	0.11	568.0	CH,S	IVS4-1 G→C/P992I
7	M	Impaired LFT	3	Undetectable	223.2	CH,S	G943D/C490X
8	M	Impaired LFT	4	Undetectable	350.8	ND	IVS4-1G→C/P992I
9	M	Impaired LFT	8	Undetectable	358.8	ND	P992L/L1181P
10	M	FH	1	ND	8274.0	FH	ND
11	M	LC	1	Undetectable	629.9	LC	ND

*Diagnostic criteria of Wilson's disease: serum ceruloplasmin <0.2 g/L, urine copper >100 μg/24hr. LFT = liver function tests; CH = chronic hepatitis; S = steatosis; LC = liver cirrhosis; FH = fulminant hepatitis; ND = not done; S/S = symptoms/signs; Dx = diagnosis.

Table 2 Mutations identified in Wilson's disease (WD) chromosomes

Mutation	Sequence	Exon	Predicted effect	Frequency of associated WD chromosomes
Missense				
G943D	GGT→GAT	12	Disrupts Tm5	3/14
R778Q	CGG→CAG	8	Disrupts Tm4	2/14
P992L	CCC→CTC	13	Disrupts cation channel and Tm6	3/14
Nonsense				
C490X	TGC→TGA	3	Truncates protein	1/14
Insertion				
2304insC	ACGCCCCCCCATG	8	Frameshift	1/14
Splicing				
IVS4 -1 G>C	gttgcagATCACA	5	Skips exon 5	3/14
Novel				
L1181P	CTG→CCG	16	Disrupts ATP binding	1/14

provided 100% sensitivity and specificity for differentiating fulminant hepatic failure caused by WD from other disorders.[15] The ratio in this case was 1.02, which was consistent with a diagnosis of WD.

3.3. Treatment and outcome of follow-up

Only Case 10 had a new Wilson index score of more than 11, and died of fulminant hepatic failure before diagnosis of WD. Case 11 had received a liver transplantation 1 month after diagnosis. All patients initially received D-PCN or trientine therapy with (1/9) or without zinc (8/9). Maintenance therapy with zinc alone began if there were no signs of hepatic decompensation. The total therapeutic duration ranged from 0.2–11.3 years (mean duration, 5.1±4.1 years). All patients showed improved liver function tests, but the asparate aminotransferase/alanine aminotransferase ratio remained elevated in three patients (Table 3).

Zinc therapy was given for 0.1–5.3 years (mean period, 3.4±2.1 years). Seven of the nine patients had serum copper levels below the normal range, and six of the eight had serum zinc levels above the upper limit. However, none had symptoms of zinc toxicity such as abdominal pain, diarrhea, nausea or vomiting, or symptoms of copper deficiency such as anemia, leukopenia or neurologic manifestations (including ataxia, neuropathy or cognitive deficits; Table 4).

4. Discussion

Although there have been several reports on children with WD in Asia,[9,10] studies that include the

Table 3 Liver function tests upon admission and after therapy

No.	Upon admission							After therapy			
	GOT/GPT (U/L)	γ-GT (U/L)	Total bilirubin (μmol/L)	WBC ($\times 10^9$/L)	INR	Albumin (g/L)	New Wilson index	GOT/GPT (U/L)	γ-GT (U/L)	Total bilirubin (μmol/L)	Duration of therapy (yr)
1	95/306	44	8.6	6.900	0.84	42	2	24/32	19	15.4	11.3
2	11/17	13	47.9	27.770	1.72	18	10	21/31	44	6.8	9.7
3	34/39	12	6.8	7.300	1.22	47	1	15/16	18	6.8	8.5
4	35/19	10	58.2	8.200	1.60	24	5	22/22	24	10.3	10.0
5	102/319	114	6.8	11.430	1.02	45	4	39/63	26	5.1	3.6
6	117/260	51	5.1	7.500	0.88	46	2	29/25	15	10.3	2.4
7	783/816	34	5.1	7.800	1.05	42	6	38/30	8	1.7	1.7
8	95/194	99	25.7	4.550	–	49	0	60/105	61	12.0	1.5
9	160/212	27	10.3	10.200	–	49	4	63/89	27	13.7	0.2
10	6736/6352	216	466.9	2.000	2.16	42	12	–	–	–	–
11	50/55	108	10.3/3.4	4.500	1.31	26	3	–	–	–	–

GOT/GPT = glutamic-oxalocetic transaminase/glutamic-pyruvic transaminase; γ-GT = γ-glutamyl transpeptidase; WBC = white blood cell; INR = international normalized ratio.

Table 4 Blood cell counts before and after therapy

Case	1	2*	3	4*	5†	6	7	8	9
Pre-therapeutic Hb (mg/L)	138	52	136	77	124	137	126	162	143
Post-therapeutic Hb (mg/L)	154	136	142	134	116	132	124	162	129
Post-therapeutic WBC ($\times 10^9$/L)	7.000	7.900	7.300	5.000	5.700	7.700	7.900	4.100	11.000
Post-therapeutic platelet ($\times 10^9$/L)	273	339	267	200	424	293	408	193	434
Serum copper‡ (μmol/L)	1.6	6.0	3.8	10.2	9.7	13.0	4.6	11.0	5.5
Serum zinc§ (μmol/L)	24.0	17.0	29.5	13.6	29.8	25.7	25.2	31.2	–
Duration of zinc therapy (yr)	5.0	5.0	5.3	6.0	3.5	2.2	1.6	1.5	0.1

*Cases 2 and 4 were patients with hemolytic anemia; †case 5 was a patient with thalassemia; ‡normal range of serum copper: 11.0–23.6 μmol/L; §normal range of serum zinc: 10.7–18.5 μmol/L. Hb = hemoglobin.

genetic diagnosis of WD are rare. Based on the diagnostic criteria of AASLD (2008),[8] WD can be diagnosed by the combination of K-F rings, low serum ceruloplasmin and high 24-hour urinary copper. If these three criteria are not fully met, molecular testing and analysis of copper levels in dry liver should be performed to confirm the diagnosis. The dry liver copper exam is invasive and not always available, and K-F rings are rare in childhood.[16] Thus, genotyping studies play an important role in the diagnosis of children with atypical features. Moreover, WD could be diagnosed solely by genetic analysis, even if the other criteria are not fulfilled.[8,17]

Early diagnosis is critically important for the treatment of WD, in order to avoid severe complications.[16] Because the symptoms caused by copper deposition develop over time, K-F rings and neurologic symptoms often do not appear until adolescence, and thus WD is difficult to diagnose in children due to its obscure clinical manifestations.[7] The most common initial presentation in children with WD is impaired liver function tests,[16] and WD presenting with hemolytic anemia is often overlooked.[18] The AASLD guidelines suggest that the first lines of screening for WD should be serum ceruloplasmin, 24-hour urinary copper, and slit-lamp examination.[8] In this study, case 1 was diagnosed as idiopathic hemolytic anemia rather than WD until 4.4 years after the onset of symptoms. However, the last patient (case 4) presenting as hemolytic anemia was diagnosed within weeks. Thus, serum ceruloplasmin and 24-hour urinary copper examinations should be

considered in patients with chronic hepatitis of unknown etiology, or hemolytic anemia. In addition, diagnosis should include early genetic screening if the clinical criteria are not fulfilled.

DNA sequencing for disease diagnosis is currently convenient and available; genetic diagnosis of WD can therefore be applied widely in asymptomatic siblings and patients with atypical features. Previous studies have shown that genes mutated in Taiwanese patients with WD were located in specific exons.[4,13] The observed mutations differed from those detected in either European or North American patients.[19,20] This finding suggests that the mutation spectrum of WD may follow a population-dependent pattern. These exons should therefore be screened initially in children in Taiwan with suspected WD, in order to shorten the detection time and decrease the cost. Early intervention could reduce the damage caused by copper deposition, as well as the incidence of end-stage liver disease. The discovery of the WD gene is thus important for opening up a new molecular diagnostic approach, which may be invaluable in the early diagnosis of WD in children with obscure clinical manifestations, and could also form the basis of future gene therapies.[21]

The currently approved treatments for WD include D-PCN, zinc acetate and trientine.[22–24] However, chelating therapy with D-PCN or trientine may be associated with a risk of central nervous system deterioration.[25] Zinc is currently the recommended therapy for long-term management of WD and presymptomatic Wilsonian patients.[26] In our experience, all patients showed improved liver function tests after therapy. Furthermore, the hemoglobin level in two patients with hemolytic anemia was restored without the need for blood transfusions. The most common reported adverse effect of zinc therapy is mild and transient gastric disturbance, and the first signs of clinical copper deficiency are anemia and leucopenia.[12] All the children in the current study tolerated zinc therapy well, even though their serum zinc levels were above the normal limit. Although all patients had low levels of serum copper, the hemoglobin levels during the follow-up period were within the normal limits, except in one patient with thalassemia. In addition, white cell and platelet counts did not show significant abnormalities. However, several reports of morbidity and mortality after zinc therapy have been noted.[27–29] Since zinc therapy is lifelong,[8] zinc excess and copper deficiency may become a problem. A previous study[30] showed that low-dose zinc administration could also be effective for the treatment of WD, and could reduce the effects due to zinc excess and copper deficiency. Further studies are required to determine the exact recommended zinc dosage for Taiwanese children with WD.

In conclusion, children with chronic hepatitis and hemolytic anemia should receive serum ceruloplasmin and 24-hour urinary copper examinations to rule out a diagnosis of WD. Prompt diagnosis can help to prevent fatal complications of WD, and genetic screening may thus be helpful in asymptomatic siblings and patients with atypical features. Long-term follow-up is required to monitor the adverse effects of zinc excess and copper deficiency. Further studies are required to establish a suitable zinc dosage for long-term treatment of pediatric WD patients.

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