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BIOCHEMICAL STAGING OF THE CHRONIC HEPATIC LESIONS OF WILSON DISEASE

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ABSTRACT

Background: Copper toxicity steadily affects the livers of patients with Wilson disease. However, the toxic effect of copper on serum aspartate and alanine aminotransferase levels remains to be clarified as a prerequisite for diagnostic tests. The clinical records of 33 cases were analyzed to clarify the natural history of Wilson disease. Phenotypes were simplified into hepatic, acute, and neurologic. The bio-low stage of both enzymes was less than 40 IU/L, the bio-moderate stage was intermediate between 40 and 200 IU/L, and the bio-high stage was more than 200 IU/L of either or both enzymes. Rebounded enzyme levels at the recovery period from anemia were presumed to be the chronic baselines when pre-anemic enzyme levels were not available in the acute phenotype. We investigated whether these enzyme levels may provide information useful for screening patients. The natural history of chronic Wilson disease consisted of the first increasing and second decreasing phases. The clinical courses of a 4-year-old boy and 12-year-old girl were representative of the 2 phases, respectively. All but one patient were in the decreasing phase. Negative correlations were obtained between age and enzyme level in the decreasing phase. The hepatic phenotype may be a prototype found throughout the 2 phases, and acute and neurologic phenotypes may be major complications in the bio-moderate and bio-low stages of the decreasing phase, respectively. Biochemical staging may provide a better understanding of Wilson disease when combined with phenotypes. Bio-high stage patients should be referred to a medical center for diagnosis.

Key Words: AST, ALT, Phenotype, Stage, Wilson disease

INTRODUCTION

Dietary copper is absorbed in the intestine and exported in the portal vein via the ATP7A protein, while circulating copper in the portal stream is preferably uptaken by hepatocytes[1)]. The hepatic copper transporter ATPase coded by the ATP7B gene exists in the late endosomes and Golgi networks of hepatocytes, and transport cytosolic copper into membranous lumens for

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cuproprotein synthesis and the physical elimination of excess copper into the bile[2]. Therefore, the ATP7B protein is one of the key enzymes of copper homeostasis. Wilson disease (WD) has been attributed to the defective function of the ATP7B protein[3]. Because the physiological secretion of copper into the bile is blocked, toxic copper remains in the hepatocyte cytoplasm of individuals exhibiting disease traits from birth. Toxic copper–induced liver pathologies are steatosis, steatohepatitis, chronic hepatitis, and cirrhosis in progression[4]. Copper toxicosis initially occurs in the liver, and later expands to extra-hepatic organs due to the steady absorption of dietary copper. Although WD is a primary liver disease, patients exhibit various clinical manifestations of a wide range of liver diseases, including fulminant hepatitis and cirrhosis, as well as hemolytic anemia and neuropsychiatric symptoms[5]. Therefore, a scoring system for the diagnosis of WD has been proposed by an international group for the study of WD[6]. ATP7B analysis is an absolute diagnostic test used in this system.

Patients with possible WD should be screened using standard liver function tests including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) prior to diagnostic testing for WD[7, 8]. A prerequisite for specific tests and ATP7B analysis may include a better understanding of the biochemical features of the primary hepatic lesions of WD. The characterization of hepatic copper toxicosis using biochemical stages combined with the phenotypes proposed in this study may allow for an early diagnosis, followed by effective treatment with anti-copper regimens.

METHODS

Patients and diagnostic ATP7B analysis

WD was diagnosed by a modified application of the international scoring system. Patients were initially screened using the scoring system with more than 3 points. Informed consent for ATP7B analysis was then obtained from each patient according to the study protocols approved by the Ethical Committees of the participating institutes (Permission ID: Nagoya University School of Medicine No. 277, Aichi Gakuin University School of Pharmacy No. 6 and 8). A long-range polymerase chain reaction was applied to the 21 exons and their boundaries of the ATP7B gene[9]. A total of 30 patients who were homozygous or compound heterozygous for ATP7B mutations were finally enrolled in this study. The medical records of 3 patients were analyzed twice; one patient was complicated by hemolytic anemia after 5 year-interruption of anti-copper treatment, while 2 patients had long observation periods between the initial visit and definite diagnosis. Our database did not include patients younger than 3 years of age, whose AST and ALT levels were referred to the literature[10, 11].

Three phenotypes of Wilson disease

The phenotypes of WD had been classified into 3 subtypes based on their clinical features: phenotype H of hepatic WD, phenotype A of acute WD including hemolytic anemia, and phenotype N of neuropsychiatric WD, which were more simplified than the International phenotypes of H1, H2, N1, N2, and Nx[6]. Most patients with phenotype H were asymptomatic and incidentally identified using blood tests. An exception was a family study intended for the siblings of WD patients. A small number of elderly patients with phenotype H were symptomatic due to decompensated cirrhosis. Phenotype A appeared with acute episodes accounted for by copper toxicosis such as hemolysis and acute hepatic failure. Hemolytic episodes were commonly associated with self-limiting anemia and jaundice. Phenotype N was complicated by acute or chronic neuropsychiatric manifestations. Underlying advanced hepatic lesions were confirmed in all patients with phenotypes A and N by either liver biopsy or abdominal imaging.

Presumed baselines of the chronic hepatic lesions of phenotype A

Because the biochemical parameters of phenotype A acutely changed during the one-month period of an anemic episode, the underlying baselines of chronic hepatic lesions were selected from either the pre-anemic or recovery periods. In the patients whose data were not available in the pre-anemic period, rebounded levels in the recovery period replaced those in the pre-anemic period based on the hypothesis that the underlying chronic hepatic lesions also appeared in the recovery period. The short-term effects of anti-copper regimens were neglected in the recovery period. These chronic hepatic lesions underlying phenotype A were used to postulate the natural history of WD.

Two phases of increasing and decreasing aminotransferase levels and three biochemical stages of chronic hepatic lesions of Wilson disease

To characterize the biochemical hepatic lesions of WD with aging, 2 standard parameters of the serum enzyme activities of AST and ALT with modification in phenotype A for underlying chronic hepatic lesions were analyzed as serum enzyme levels per se and AST and ALT profiles at the onset ages of patients. Based on the data of neonates and a baby obtained from literature[10, 11] and our patients, the natural history of biochemical parameters was divided into the first increasing phase and second decreasing phase of AST and ALT levels. Liver histology also provided information for phasing: steatosis was found in the increasing phase, while steatohepatitis, chronic hepatitis, and cirrhosis were observed in the decreasing phase. Using AST and ALT levels, biochemical parameters were divided into 3 stages. The biochemical low (bio-low) stage was lower than 40 IU/L (the normal upper level) in both enzyme activities, while the bio-moderate stage was intermediate between 40 and 200 IU/L, and the bio-high stage was higher than 200 IU/L (5 times the normal upper level) in either or both enzyme activities. As far as the bio-high stage was concerned, it was not only clinically important, but also actually difficult to classify a patient phase. Data cited from the literature and case record of a 4-year-old patient in the increasing phase were removed from statistical analysis to visualize the natural features of the decreasing phase, while all patient data at the bio-high stage were included in the decreasing phase.

Statistics

The onset ages and biochemical parameters of patients in the 3 phenotypes and 3 stages were expressed as the mean±SD, and differences were examined using the Student's t-test. $P<0.05$ was considered significant. Correlations were assessed between the onset ages and serum levels of AST and ALT in patients in the decreasing phase.

RESULTS

Clinically, 17 patients had phenotype H, 7 phenotype A, and 9 phenotype N (Table 1). All patients were residents in central Japan. The mean ages of patients with phenotype H was 12±6 years, while those of patients with phenotypes A and N were 17±10 years and 19±7 years, respectively. A significant difference was observed in the onset ages of patients between phenotypes H and A, and H and N ($p<0.05$). WD is autosomal recessive in its inheritance; however, reasons for the male dominancy observed in our patients remain unknown.

Anemia and jaundice in phenotype A were self-limiting in all but one patient. One of the 7 patients died from acute hepatic failure. The biochemical parameters of 6 survivors showed marked changes during a short-term observation period (Table 2). The pre-anemic levels of

Table 1 Clinical Features of Patients with the 3 Phenotypes on Admission

Phenotype H Patient	Age[a] (yrs)	Gender[b]	Residence (prefecture)	ALT (IU/L)	*ATP7B* mutation-1	*ATP7B* mutation-2
1	4	M	Aichi	197	2333G>T	3104G>T
2	5	M	Aichi	725	2299insC	2307G>T
3	5	M	Ishikawa	789	2755C>G	3809A>G
4	8	M	Aichi	410	1708-5T>G[c]	2333G>T
5	8	M	Aichi	436	2333G>T	3104G>T
6	10	M	Ishikawa	89	2333G>T	3029A>C
7	10	M	Aichi	39	2333G>T	2333G>T
8	11	F	Gifu	238	2333G>T	2871delC
9	12	F	Aichi	22	2871delC	3809A>G
10	12	F	Aichi	218	2333G>T	2621C>T
11	13	M	Aichi	51	2871delC	3643G>T
12	16	M	Aichi	357	453delC	2871delC
13	16	M	Aichi	116	1708-5T>G[c]	1708-5T>G[c]
14	17	M	Aichi	90	2333G>T	2621C>T
15	18	M	Aichi	38	1708-5T>G[c]	1708-5T>G[c]
16	21	F	Gifu	23	1708-5T>G[c]	2333G>T
17	23	M	Mie	30	3664G>A	3664G>A
	12±6[d]	F/M=4/13		228±242[d]		
Phenotype A Patient	Age[a] (yrs)	Gender[b]	Residence (prefecture)	ALT (IU/L)	*ATP7B* Mutation-1	*ATP7B* Mutation-2
1	6	F	Aichi	185	2333G>T	2333G>T
2	9	F	Aichi	127	2785A>G	3104G>T
3	11	M	Gifu	22	3787delG	3787delG
4	17	M	Aichi	10	2871delC	3643G>T
5	18	F	Aichi	21	2621C>T	2650del3
6	24	M	Aichi	179	1708-5T>G[c]	1708-5T>G[c]
7	36	F	Aichi	43	3800A>C	3837bpdel in exon5-9
	17±10[d]	F/M=4/3		84±78[d]		
Phenotype N Patient	Age[a] (yrs)	Gender[b]	Residence (prefecture)	ALT (IU/L)	*ATP7B* Mutation-1	*ATP7B* Mutation-2
1	13	F	Ishikawa	34	2975C>T	3086C>T
2	13	M	Aichi	16	2632C>T	2871delC
3	16	M	Aichi	27	2871delC	3664G>A
4	17	M	Aichi	16	2871delC	3809A>G
5	17	M	Aichi	20	2871delC	2871delC
6	18	M	Aichi	13	1708-5T>G[c]	3809A>G
7	19	M	Gifu	46	2332C>T	3029A>C
8	23	M	Aichi	19	2333G>T	3556G>A
9	37	M	Ishikawa	19	2332C>T	2755C>G
	19±7[d]	F/M=1/8		23±11[d]		

a) The onset ages of phenotype H were different from those of phenotype N.
b) ATP7B-Wilson disease is autosomal recessive in its inheritance; however, reasons for the male dominancy observed in our patients remain unknown.
c) 1708-5T>G; One exon skipping in the coding region as reported by Shimizu *et. al.*[8]
d) Expressed as mean±SD.
F; female, M; male.

Table 2 Acute Changes in the Biochemical Parameters of Patients with Phenotype A

	Pre-anemic (n=3)	Anemic (n=6)	Recovery (n=4)	Chronic (n=7)
AST (IU/L)	122±60	77±30	136±38	126±47
ALT (IU/L)	136±80	26±14	124±33	125±53
Hb (g/dL)	14.2±2.3	8.3±3.1	12.4±0.5	13.3±1.4
Bilirubin (mg/dL)	1.2±0.2	9.1±2.9	1.4±1.0	1.3±0.8

All 4 parameters changed during acute episodes of hemolytic anemia.
Anemia and jaundice disappeared in one month in all, but one patient. One patient died from acute hepatic failure.
It is important to note that the underlying hepatic lesions in phenotype A were modified by these acute changes. Pre-anemic biochemical parameters (Pre-anemic) were available in 3 patients. Based on the hypothesis that rebounded levels of AST and ALT in the recovery period (Recovery) may represent aspects of chronic hepatic lesions, chronic hepatic lesions (Chronic) were finally expressed by pre-anemic biochemical parameters for 3 patients and rebounded levels of biochemical parameters in the recovery period for 4 patients. These chronic hepatic lesions gradually responded to anti-copper regimens, except for one patient who died.

ALT were 136±80, anemic levels 26±14, and recovery levels 124±33. The reduction in the anemic levels of AST was milder than that of ALT. The rebounded levels of AST and ALT were presumed to be the baselines underlying phenotype A patients. The chronic hepatic lesions of either pre-anemic enzyme levels or presumed baselines at recovery from anemia gradually improved with anti-copper regimens.

The onset ages and ALT levels modified for chronic hepatic lesions were shown in Figure 1, in which data was cited from the literature. The 4-year natural history of a 4-year-old boy, and 9-year natural history of a 12-year-old girl were drawn by 2 points, while the ALT levels of other patients were by one point. The pattern of AST levels was similar to that of ALT levels. The serum levels of AST and ALT revealed the first increasing phase and second decreasing phase, and the histories of a 4-year-old boy and 12-year-old girl were representative of the increasing phase and decreasing phase, respectively. All but one patient were in the decreasing phase. A 4-year-old boy with phenotype H was in the increasing phase due to steatosis in liver pathology. A 6-year-old girl with phenotype A was in the decreasing phase complicated by histological pre-cirrhosis as reported elsewhere[12].

Negative correlations were found between the onset ages and enzyme levels of chronic hepatic lesions in patients in the decreasing phase (Table 3). The best coefficient 0.54 was found between the serum levels of ALT and ages of patients with phenotype H (Figure 2). The coefficient R^2 between ALT levels and ages of all patients was 0.29. Similar results were obtained for AST levels.

As summarized in Table 4, the ages and AST and ALT levels of chronic hepatic lesions were different, whereas Hb and serum bilirubin concentrations were not among the 3 biochemical stages. As shown in Figure 3, all patients in the bio-high stage were ALT-dominant in AST and ALT profiles and exhibited phenotype H without any complications. Their AST and ALT levels did not change without anti-copper regimens, but responded to specific treatments. ALT-dominancy was lost in patients in the bio-moderate stage. The major phenotypes in the bio-moderate stage were H and A. The majority of patients in the bio-low stage exhibited phenotype N, while the

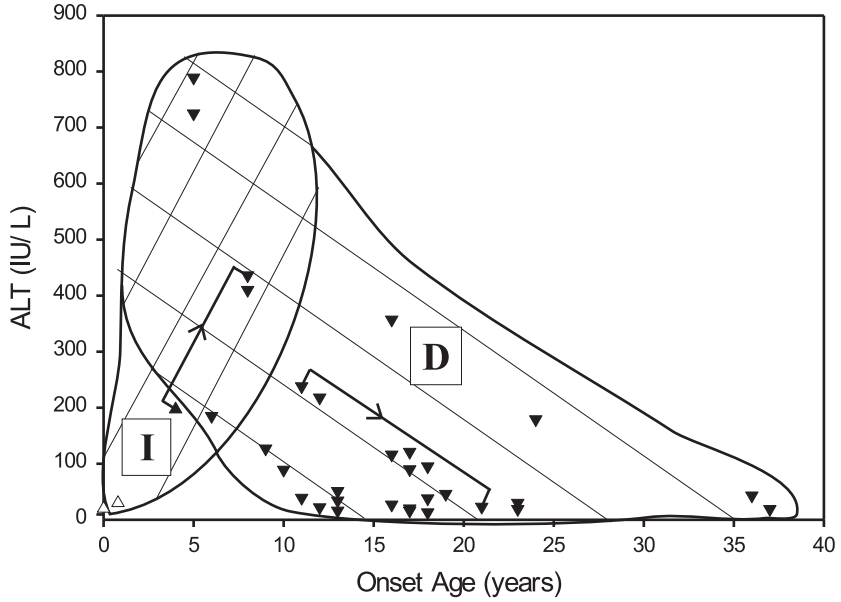

Fig. 1 Natural history of chronic hepatic lesions of patients with Wilson disease

Associations between the onset ages of patients and ALT levels as indicators of chronic hepatic lesions are illustrated in this figure. Data from neonates and a baby are cited from the literature. Two of our patients underwent long-term observation without copper chelation. Two time-point ALT levels (at initial visit and definite diagnosis) were employed for these patients, while ALT levels at the initial visit were employed for the other patients. The enzyme levels of phenotype A were replaced by those of presumed chronic hepatic lesions.

ALT levels were normal in neonates[10], and remained at 30 IU/L in an 8-month-old patient[11]. Enzyme levels increased from 197 to 436 IU/L during a 4-year non-specific treatment period in a 4-year-old boy, while these decreased from 236 to 23 IU/L during a 9-year period in a 12-year-old girl, as shown by straight lines. The increasing and decreasing phases of patients younger than 10 years old were also determined by liver histology.

The serum levels of ALT revealed the first increasing phase (I) and second decreasing phase (D).

Regarding our patients were concerned, all but one were in the decreasing phase. The 4-year-old boy was found to be in the increasing phase and complicated by steatosis of the liver, while a 6-year-old patient whose liver histology was pre-cirrhosis was classified in the decreasing phase. The ALT changes of the 4-year-old boy and 12-year-old girl are representative of the increasing and decreasing phases, respectively. It is likely that ALT levels change from an increasing to decreasing phase with the increasing onset age of patients. It was difficult to classify the phase in the bio-high stage, so patients in this stage were classified in the decreasing phase for statistical analysis as shown in Figure 2.

△; ALT levels in the increasing phase cited from the literature, ▲; the ALT level of a 4-year-old patient in the increasing phase, ▼; the ALT levels of patients in the decreasing phase.

minority was phenotype H. No characteristics of biochemical parameters were found in the bio-low stage.

DISCUSSION

Genetic copper toxicosis is always progressive in individuals with ATP7B-WD traits[1]. However, according to the literature[10, 11] and clinical records of our patients, the natural profiles of AST and ALT, which are biochemical markers of liver cell necrosis, revealed the first increasing phase

Table 3 Ages and Biochemical Parameters of Patients with the 3 Stages of Chronic Hepatic Lesions in the Decreasing Phase

	Age[a)] (yrs)	AST (IU/L)	ALT (IU/L)	Hb[b)] (g/dL)	Bilirubin[b)] (mg/dL)
Bio-High Stage (n=7; H/A/N=7/0/0)					
Mean±SD	8±3	308±163	469±240	13.1±0.8	1.0±0.4
Bio-Moderate Stage (n=15; H/A/N=7/6/2)					
mean±SD	16±7	80±48	84±53	13.1±1.3	0.9±0.6
p; v bio-high S.	<0.01	<0.01	<0.01	nd	nd
Bio-Low Stage (n=9; H/A/N=2/0/7)					
mean±SD	20±7	24±7	21±8	13.4±2.2	0.8±0.1
p; v bio-high S.	<0.01	<0.01	<0.01	nd	nd
p; v bio-moderate S.	nd	<0.01	<0.01	nd	nd

Data of a 4-year-old patient in the increasing phase were removed from the analysis.
a) Onset ages of patients in the bio-high stage were different from those in the other stages.
b) Serum levels of Hb and bilirubin in patients in the bio-moderate stage were similar to those in the other stages.
H; phenotype H, A; phenotype A, N; phenotype N, nd; no difference.

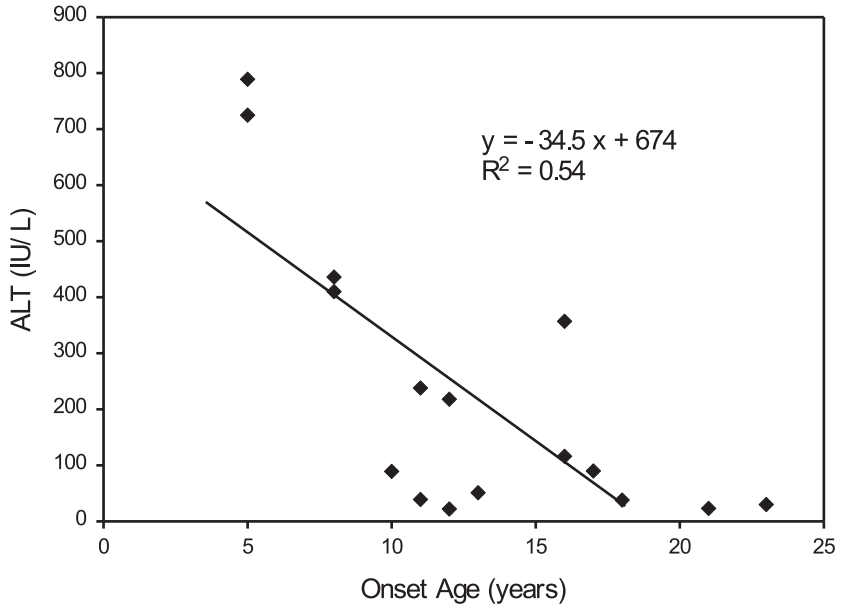

Fig. 2 A negative correlation between the onset ages and ALT levels of patients with phenotype H in the decreasing phase
It is important to note that the second phase of progressive copper toxicosis is camouflaged by misleading ALT levels that gradually decrease with age.

Table 4 Correlations between the ages and aminotransferase levels of patients in the decreasing phase

	Regression Line	R^2
Ages and ALT Levels		
Phenotype H Alone	ALT = –34.5 × Age + 674	0.54
All Phenotypes	ALT = –14.3 × Age + 374	0.29
Ages and AST Levels		
Phenotype H Alone	AST = –21.4 × Age + 432	0.50
All Phenotypes	AST = –9.4 × Age + 261	0.31

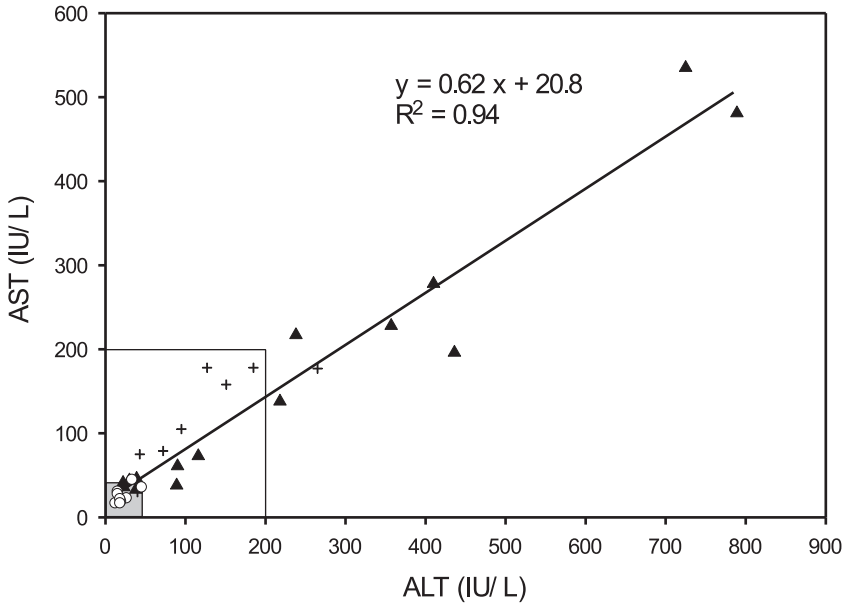

Fig. 3 Chronic hepatic lesions of the 3 phenotypes in the decreasing phase presented by AST and ALT profiles
Enzyme levels of phenotype A were modified for underlying chronic hepatic lesions. Phenotype H was distributed widely in the 3 stages, while most of phenotypes A and N were localized in the bio-moderate and bio-low stages, respectively.
The bio-low stage was lower than 40 IU/L (the normal upper level) in both AST and ALT activities, while the bio-moderate stage was intermediate between 40 and 200 IU/L in either enzymes, and the bio-high stage was higher than 200 IU/L (5 times the normal upper level) in either or both enzyme activities.
▲; phenotype H, +; phenotype A, ○; phenotype N. The bio-low stage is shaded.

and second decreasing phase. The increasing phase may not be clinically important because no special treatment is needed in this phase. An 8-year-boy who was found to be in the increasing phase at the age of 4 years had an excellent prognosis after an anti-copper regimen. Therefore, we focused on the natural history of WD in the decreasing phase.

To better understand the natural history of WD, special attention should be paid to the acute complications associated with phenotype A. One of the 7 patients died from acute hepatic failure. Other patients survived acute episodes, but showed marked changes in their biochemical param-

eters during a one-month period. These changes were closely related to severe anemia. Similar findings were reported in Germany[13]. Serum AST and ALT levels were only moderately elevated with a markedly reduction in ALT activity in 2 WD patients with non-autoimmune hemolysis. Although data on the pre-anemic period were limited in our patients, rebounded levels of AST and ALT that appeared in the recovery period were representative of the chronic baselines of hepatic damage underlying phenotype A.

The biochemical parameters of copper-induced hepatotoxicity in the second phase may be masked by disease progression-dependent modifiers. Serum AST and ALT levels generally decrease with progression from chronic hepatitis to cirrhosis regardless of etiologies. At least 2 specific modifiers have been identified in copper toxicosis. One is a detoxification system by metallothionein induction and lysosomal storage. Lysosomal proliferation visualized by histochemistry for copper proteins was poor in steatohepatitis with high enzyme activities, and strongly positive in cirrhotic livers camouflaged by normal enzyme levels[14]. A large amount of copper detoxified to cuprothioneins was safely stored in the lysosomal system of the cirrhotic livers of WD[15]. The other modifier is shunt formation in the copper-rich portal stream from the liver to extra-hepatic organs via intra- and extra-hepatic collateral circulation. Ammonia-induced encephalopathy is well-known in cirrhosis[16]; however, the effect of collateral circulation has not yet been investigated in WD. Intra- and extra-hepatic portal shunts may induce the extra-hepatic distribution of toxic copper, resulting in the later development of general copper toxication including extrapyramidal signs and Kayser Fleisher rings. The hypothesis that the daily amount of copper directly reaching the liver may be reduced in WD patients with cirrhosis needs to be clarified in future studies.

The chronic hepatic lesions observed in our patients changed from the bio-high stage to low stage in the second phase. These biochemical stages in the decreasing phase were age-dependent. Based on these findings, we speculated that all patients with WD traits fundamentally have phenotype H with biochemical transform from the increasing to decreasing phase, and some patients may be complicated by phenotype A and phenotype N in the bio-moderate and bio-low stages of the decreasing phase, respectively. Onset ages were also different between phenotype H and other phenotypes, but the first symptom of phenotype A was observed in a 6-year-old girl in the bio-moderate stage of the decreasing phase[12], which suggested that biochemical staging rather than the age of patient may be a better time limit for instituting anti-copper treatments.

Most patients with phenotype H in the bio-high stage have been characterized by a young age, ALT-dominant hepatic damage, and an absence of the signs of extra-hepatic organ damage. The response to anti-copper regimens was excellent in these patients in spite of high liver enzyme activities. In contrast, the prognosis of patients with phenotypes A and N has not always been so good[5]. Some patients with phenotype A may be complicated by acute hepatic failure, and patients with phenotype N may have serious central nervous system damage.

CONCLUSIONS

To understand the natural history of WD, transient changes during hemolytic anemia were replaced by chronic hepatic lesions underlying phenotype A. Serum AST and ALT levels in patients in the second phase gradually decreased with the onset age as well as misleading progression from the bio-high to low stage. Most patients with phenotype A appeared in the bio-moderate stage, while most patients with phenotype N appeared in the bio-low stage of the decreasing phase. Patients with phenotype H in the bio-high stage should preferentially be referred to liver centers to undergo diagnostic tests.

Conflict of Interest: None

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