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Zinc monotherapy for young children with presymptomatic Wilson disease: a multicenter study in Japan

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Abstract

Background and Aim: Few studies of zinc monotherapy for presymptomatic Wilson disease have focused on young children. We therefore evaluated long-term efficacy and safety of zinc monotherapy for such children, and established benchmarks for maintenance therapy.

Methods: We retrospectively and prospectively examined children under 10 years old with presymptomatic Wilson disease who received zinc monotherapy from time of diagnosis at 12 participating pediatric centers in Japan.

Results: Twenty-four patients met entry criteria. Aspartate aminotransferase and alanine aminotransferase decreased significantly beginning 1 month after initiation of treatment and usually remained under 50 U/L from 1 to 8 years of treatment. Twenty four-hour urinary copper decreased significantly at 6 months, and usually remained under 75 μg/day and between 1 and 3 μg/kg/day for the remainder of the study. All patients continued to take zinc, and none became symptomatic. In patients under 6 years old who received 50 mg/day of zinc as an initial dose, aspartate aminotransferase and alanine aminotransferase significantly decreased at 1 month after initiation of treatment, as did γ-glutamyltransferase and 24-hour

urinary copper at 6 months.

Conclusions: To our knowledge, this is the first multicenter study of zinc monotherapy for young children with presymptomatic Wilson disease. Such monotherapy proved highly effective and safe. Maintaining normal transaminase values (or values under 50 U/L when normalization is difficult) and 24-h urinary copper excretion between 1 and 3 μg/kg/day and under 75 μg/day is a reasonable goal. An initial dose of 50 mg/day is appropriate for patients under 6 years old.

Key words: Wilson disease, young children, zinc, presymptomatic

Introduction

Wilson disease (WD) is an autosomal recessive disorder of copper metabolism caused by mutations in the *ATP7B* gene leading to copper accumulation in hepatocytes and in extrahepatic organs such as the brain and kidney. The phenotype of WD is designated by the major symptomatic organ (if any), such as "hepatic type," "neurologic type," and "presymptomatic type." The American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) guidelines recommend zinc monotherapy as treatment for presymptomatic patients with WD as well as those with neurologic WD.[1,2] In Japan, zinc acetate was accepted as therapy by the Ministry of Health, Labor, and Welfare in 2008. Zinc monotherapy is effective for presymptomatic WD, as is D-penicillamine.[3] Additionally, some reports indicate that zinc monotherapy is safer for children with presymptomatic WD than D-penicillamine.[4] However, most previous reports describe zinc monotherapy for adults and for children over 10 years old,[5-7] with less investigation of young children with presymptomatic WD.[8-10] Some previous reports recommended that patients taking zinc should maintain 24-h urinary copper excretion values under 75 to 100 μg/day,[1,2] which may represent insufficient reduction of copper in the body in treating young children with presymptomatic WD. We previously suggested that a reasonable goal in treating young presymptomatic children with WD using zinc would be maintaining 24-h urinary copper excretion between 1 and 3 μg/kg/day for the first 1 to 2 years.[10]

The AASLD and EASL guidelines recommend initial zinc doses of 150 mg/day for larger children and adults and 75 mg/day for smaller children (below 50 kg in body weight).[1,2] On the other hand, Brewer *et al.* and Shimizu *et al.* recommend an initial zinc dose of 50 mg/day for patients 1 to 5 years old and 75 mg/day for those 6 to 15 years old.[11,12] However, we know of no reports focusing on the effects of 50 mg/day as an initial dose of zinc for 1- to 5-year-old patients.

We therefore evaluated long-term efficacy and safety of zinc monotherapy for children under 10 years old with presymptomatic WD at Japanese pediatric centers, aiming to establish appropriate benchmarks for maintenance therapy. We also clarified the effect of zinc at the initial dose of 50 mg/day given to patients under 6 years old.

Patients and Methods

Between 2008 and 2016, at 12 participating pediatric centers in Japan, we retrospectively and prospectively examined young children under 10 years old with presymptomatic WD who satisfied diagnostic criteria according to the Leipzig score (≥ 4).[1,13] Presymptomatic patients are diagnosed before developing clinical manifestations of WD such as jaundice, hepatomegaly, or neurologic abnormalities--usually as a result of family screening. Occasionally, they are identified when results of routine blood tests obtained while treating respiratory infections or enteritis include elevated serum transaminases.[1,2,11] We included only

patients treated with zinc monotherapy from time of diagnosis. We monitored serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), free copper (non-ceruloplasmin-bound copper = total copper - ceruloplasmin x 3.15), and 24-h urinary copper before and after initiation of zinc monotherapy. Additional laboratory monitoring included white blood cell count, hemoglobin, platelet count, serum total bilirubin, albumin, iron, amylase, lipase, zinc, and prothrombin time, as well as 24-h urinary zinc excretion. Additionally, we performed abdominal ultrasonograpy and evaluated clinical WD manifestations, drug compliance, and adverse effects of zinc. The prescribed initial dose of zinc acetate for patients under 6 years old was 50 mg/day; for those 6 years or older, this dose was 75 mg/day.[11,12] We increased the dose of zinc if patients showed ALT elevation to more than twice the upper limit of normal, and decreased it if they showed adverse effects of zinc such as iron-deficiency anemia or pancytopenia. We evaluated patient compliance with zinc monotherapy by interview and by laboratory values for serum and urinary zinc, excluding patients poorly compliant with zinc monotherapy from the study (those suspected to have taken under 70% of prescribed doses).

To evaluate the effect of zinc at the initial dose of 50 mg/day given to patients under 6 years old, laboratory values including AST, ALT, GGT, and 24-h urinary copper were examined in these patients for the first 6 months after initiation of treatment.

The study protocol complied with the ethical guidelines of the Declaration of Helsinki of

1975 (2004 revision) and was approved by the Ethics Committee of Kurume University and other participating centers.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) and categorical variables as frequency; ranges are given in some instances. Statistical analyses were performed using Student's *t* test, analyses of variance (ANOVA), or a Tukey-Kramer test as appropriate. All statistical analysis was performed using GRAPHPAD PRISM version 6.05 software (GraphPad Software, San Diego, CA, USA). Tests were two- sided, and *P* values below 0.05 were considered to indicate statistical significance.

Results

Patient characteristics and laboratory findings at time of diagnosis

Twenty-four patients were enrolled in this study; 13 were girls. Mean age and mean body weight at diagnosis were 6.1 years (range, 3 to 9) and 21.8 kg (range, 15.6 to 34.0), respectively. Five patients were diagnosed by family screening, while 19 others were found to have unexpected laboratory abnormalities such as AST and ALT elevations when evaluated for respiratory infection or enteritis. The mean follow up period was 3.8 years (range, 0.5 to 8). No patient had a Kayser-Fleischer ring or had brain abnormalities detected by magnetic

resonance imaging (Table 1). At time of diagnosis, AST/ALT, GGT and 24-hour urinary copper were 153 ± 115/243 ± 151 U/L (mean ± SD), 98 ± 69 U/L and 118 ± 50 μg/day (5.5 ± 2.7 μg/kg/day), respectively (Table 2).

Course of clinical and laboratory values before and after initiation of zinc monotherapy

AST/ALT significantly decreased to 49 ± 13/70 ± 29 U/L (P<0.05) at only 1 month after initiation of treatment; these mostly remained under 50 U/L between 1 and 8 years (AST/ALT, 34 ± 6/40 ± 18 and 26 ±2 /30 ± 5 U/L at 1 and 8 years of treatment, respectively). Proportions of patients with normal values for both AST and ALT (≤ 30 U/L) were 18%, 41%, and 75% at 1, 2, and 8 years of treatment, respectively. GGT significantly decreased to 57 ± 49 U/L (P<0.05) at only 1 month and remained nearly normal between 1 and 8 years of treatment (Fig. 1a-c). Twenty four-hour urinary copper significantly decreased to 47 ± 19 μg/day (2.2 ± 1.0 μg/kg/day; P<0.05) at 6 months and been mostly remained under 75 μg/day and between 1 and 3 μg/kg/day for the remainder of the study (2.2 ± 0.6 and 1.4 ± 0.2 μg/kg/day at 1 and 8 years, respectively; Fig. 2a,b). Serum free copper was nearly normal during the follow-up period (Table 2 and Fig. 1d). Serum total bilirubin and amylase were normal and did not show significant change (Table 2 and Fig. S1A,B). Serum zinc significantly increased to 210 ± 80 μg/dL (P<0.05) at 1 month after initiation of treatment (Fig. S1C). All patients continued to take zinc without any evidence of toxicity or need for other drugs such as D-penicillamine or

trientine, although some patients needed to increase the amount of zinc to doses of 75 to 150 mg/day during the follow-up period. None of our 24 patients became clinically symptomatic.

Course of laboratory values in patients under 6 years old with initial dose of zinc 50 mg/day for the first 6 months

Eleven patients were diagnosed when under 6 years old; 7 were girls. Mean age and mean body weight at diagnosis were 4.5 years and 17.7 kg, respectively. At time of diagnosis, AST/ALT, GGT and 24-h urinary copper were 165 ± 157/220 ± 193 U/L, 81 ± 70 U/L, and 105 ± 60 μg/day (6.0 ± 3.7 μg/kg/day), respectively. All of these patients received an initial zinc dose of 50 mg/day during the first 6 months of follow-up. AST/ALT significantly decreased to 49 ± 13/65 ± 32 U/L at only 1 month after initiation of treatment. GGT and 24-h urinary copper significantly decreased to 23 ± 13 U/L and 43 ± 22 μg/day (2.3 ± 1.2 μg/kg/day) at 6 months after initiation of treatment, respectively (Fig. 3).

Discussion

To evaluate long-term efficacy and safety of zinc monotherapy for young children with presymptomatic WD, we retrospectively and prospectively examined 24 patients at 12 participating pediatric centers in Japan. Long-term zinc monotherapy for young children with presymptomatic WD proved highly effective and safe. An initial dose of zinc 50 mg/day was

effective for patients under 6 years old.

While AASLD and EASL guidelines recommend zinc monotherapy as a treatment for presymptomatic patients with WD,[1,2] other drugs also are available for the treatment of WD in Japan, including D-penicillamine and trientine. All three drugs are fully efficacious when taken properly, and will remove excess copper and prevent its reaccumulation. Side effects of zinc such as gastrointestinal symptoms have developed in about 54% of patients treated for WD, but these side effects were considered mild in all patients and of little consequence.[5,12] Zinc is effective and safe for children with presymptomatic WD,[8-10,14] and effectiveness of long-term treatment with D-penicillamine *versus* zinc was similar in patients who were able to continue the initial therapy.[6] On the other hand, zinc has been reported to remove copper more slowly than the other drugs.[15]

As most previous studies of zinc monotherapy have concerned adults or children over 10 years old,[5-7] we presently evaluated zinc monotherapy in children with presymptomatic WD who were under 10 years old, aiming to complement the few existing reports of young children.[8-10] Our report also describes a multicenter investigation, while the previous reports of young children were single-center studies.[8-10] All patients continued to take zinc without any evidence of zinc toxicity or need for additional drugs such as D-penicillamine or trientine. None of our 24 patients became clinically symptomatic. These results indicate that long-term zinc monotherapy for young children with presymptomatic WD is highly effective and safe.

Chelating agents rapidly decrease free copper concentrations by the formation of excretable complexes, while zinc primarily reduces the intestinal absorption of copper. In the liver, zinc also induces activity of metallothionein, which sequesters excessive copper, contributing to a mild negative copper balance.[3,16]

In all patients, the duration of initial therapy depends upon the drug being used, representing about 2 months with D-penicillamine or trientine and about 4 to 6 months with zinc monotherapy.[15] However, in single-center studies, Mizuochi *et al.* and Abuduxikuer *et al.* reported that zinc monotherapy for children with presymptomatic WD significantly decreased AST and ALT in 1 month after initiation of zinc and reduced 24-h urinary copper excretion over 6 to 8 months.[8,10] In our multicenter study, AST and ALT significantly decreased, most often to under 100 U/L, at 1 month after initiation of zinc. Additionally, 24-h urinary copper had significantly decreased, usually to under 75 μg/day, at 6 months after initiation of zinc. These results indicate that zinc monotherapy for young children with presymptomatic WD could reverse copper accumulation somewhat faster than previously reported.

The AASLD and EASL guidelines recommend that all patients with WD taking zinc should maintain 24-h urinary copper excretion values under 75 to 100 μg/day.[1,2] However, this goal may be insufficient in treating presymptomatic young children with WD. We previously reported that zinc treatment in such children should aim to maintain 24-h urinary copper excretion between 1 and 3 μg/kg/day for the first 1 to 2 years.[10] Serum free copper also can be

used to monitor zinc efficacy. Brewer *et al*. reported that a serum free copper value of 25 μg/dL or less can be considered a reasonable goal in adults undergoing zinc treatment.[17] Zinc therapy in children with WD also caused statistically significant reduction of serum free copper from pretreatment values at 1 year after initiation of zinc.[14] In this multicenter study, we maintained AST/ALT under 50 U/L and 24-h urinary copper under 75 μg/day and between 1 and 3 μg/kg/day for 1 to 8 years. None of our 24 patients became clinically symptomatic or had severe side effects from zinc. However, most serum free copper values were within the normal range, with no signifficant differences noted throughout the follow-up period. Considering pertinent results together, we suggest that a reasonable goal in treating young children with presymptomatic WD using zinc would be maintaining both transaminases at normal values (or values under 50 U/L if normalization is difficult) and 24-h urinary copper excretion under 75 μg/day and between 1 and 3 μg/kg/day. A previous report made the point that some patients with WD have normal values of serum free copper before treatment,[18] and in our study most patients had normal values during the follow-up period. Serum free copper may be insufficiently sensitive for monitoring zinc efficacy in young children with presymptomatic WD.

The AASLD and EASL recommendations include an initial zinc dose of 75 mg/day for smaller children (under 50 kg in body weight).[1,2] On the other hand, Brewer *et al.* and Shimizu *et al.* reported use of initial doses of zinc of 50 mg/day for 1- to 5-year-old patients

and 75 mg/day for 6- to 15-year-olds.[11,12] However, we know of no reports focusing on the effects of 50 mg/day as an initial dose of zinc for 1- to 5-year-old patients. In the present study, we examined laboratory values in patients under 6 years old who received 50 mg/day of zinc as an initial dose for the first 6 months of treatment. AST and ALT significantly decreased at 1 month after initiation of treatment, as did GGT and 24-h urinary copper at 6 months after initiation. These results indicate that an initial dose of zinc with 50 mg/day seems to be an appropriate dose for under 6-year-old patients with presymptomatic WD.

An important limitation of this study is the retrospective nature of data collection in most patients. As a consequence, data were acquired out at different times in individual patients. Further, the number of patients was small. A larger, more long-term prospective study would be desirable.

In conclusion, we believe this study to be the first multicenter evaluation of zinc monotherapy for young children with presymptomatic WD. Long-term zinc monotherapy for young children with presymptomatic WD is highly effective and safe. A reasonable goal in treating young children with presymptomatic WD using zinc appears to be maintaining both normal transaminase values (or values under 50 U/L if normalization is difficult) and 24-h urinary copper excretion between 1 and 3 μg/kg/day, as well as under 75 μg/day. An initial zinc dose of 50 mg/day is appropriate for patients under 6 years old with presymptomatic WD.

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Figure legends

Figure 1. Course of blood test results before and after zinc monotherapy.

(a) aspartate aminotransferase (AST), (b) alanine aminotransferase (ALT), (c) γ-glutamyltransferase (GGT) and (d) serum free copper. *, $P < 0.05$ versus Pre, **, $P < 0.01$ versus Pre; ***, $P < 0.001$ versus Pre. mo, month(s) after initiation of zinc therapy; n, number of patient at each time points; Pre, pretreatment; SD, standard deviation; y, year(s) after initiation of zinc therapy.

Figure 2. Course of urine test results before and after zinc monotherapy.

(a) 24-h urinary copper and (b) 24-h urinary copper per weight. *, $P < 0.05$ versus Pre; **, $P < 0.01$ versus Pre; ***, $P < 0.001$ versus Pre. mo, month(s) after initiation of zinc therapy; n, number of patient at each time points; Pre, pretreatment; SD, standard deviation; y, year(s) after initiation of zinc therapy.

Figure 3. Course of laboratory results before and after zinc monotherapy in patients under 6 years old receiving a 50 mg/day dose.

(a) aspartate aminotransferase (AST), (b) alanine aminotransferase (ALT), (c) γ-glutamyltransferase (GGT), (d) 24-h urinary copper, and (e) 24-h urinary copper per weight. *, $P < 0.05$ vs. Pre, **, $P < 0.01$ vs. Pre. mo, month(s) after initiation of zinc therapy; n,

number of patient at each time points; Pre, pretreatment; SD, standard deviation.

Table 1. Patient characteristics at time of diagnosis (n=24)

Gender (male/female)	11/13
Age, years, mean (range)	6.1 (3–9)
Body weight, kg, mean (range)	21.8 (15.6–34.0)
Diagnosis suspected from	
Unexpected laboratory abnormalities	19
Family screening	5
Follow up period, years, mean (range)	3.8 (0.5–8)
Kayser-Fleischer ring	0
Brain MRI abnormalities	0

MRI, magnetic resonance imaging; n, number of patients.

Table 2. Laboratory findings at time of diagnosis

		mean ± SD	reference range
AST (n=24)	(U/L)	153 ± 115	13–30
ALT (n=24)	(U/L)	243 ± 151	7–30
GGT (n=23)	(U/L)	98 ± 69	9–32
Total bilirubin (n=22)	(mg/dL)	0.6 ± 0.2	0.4–1.2
Serum ceruloplasmin (n=24)	(mg/dL)	5.6 ± 4.9	20–40
Serum free copper (n=24)	(μg/dL)	10.5 ± 7.6	<15
Urinary copper (n=20)	(μg/day)	118 ± 50	13–33
	(μg/kg/day)	5.5 ± 2.7	unknown

AST, aspartate aminotransferase; ALT alanine aminotransferase: GGT, γ-glutamyltransferase;

n, number of patients; SD, standard deviation.

Figure 1

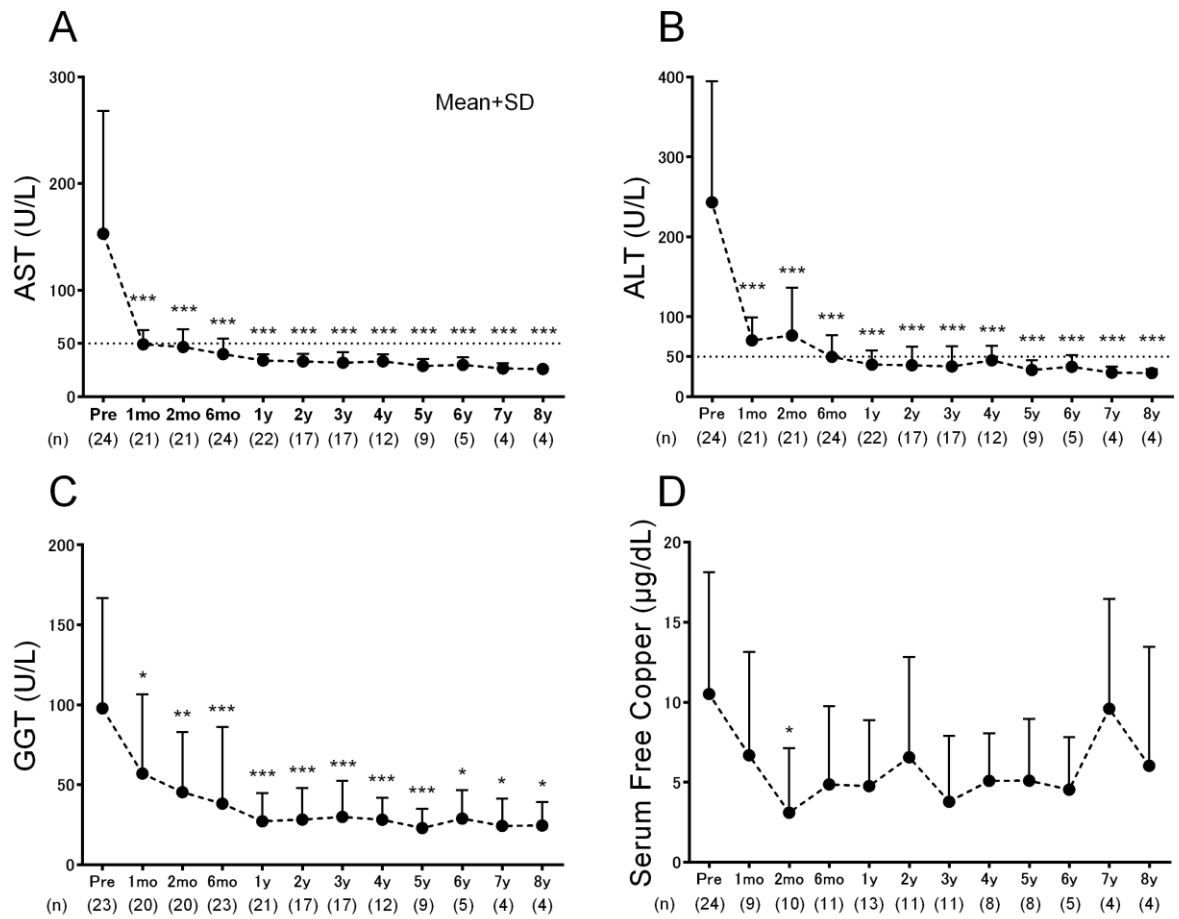


Figure 2

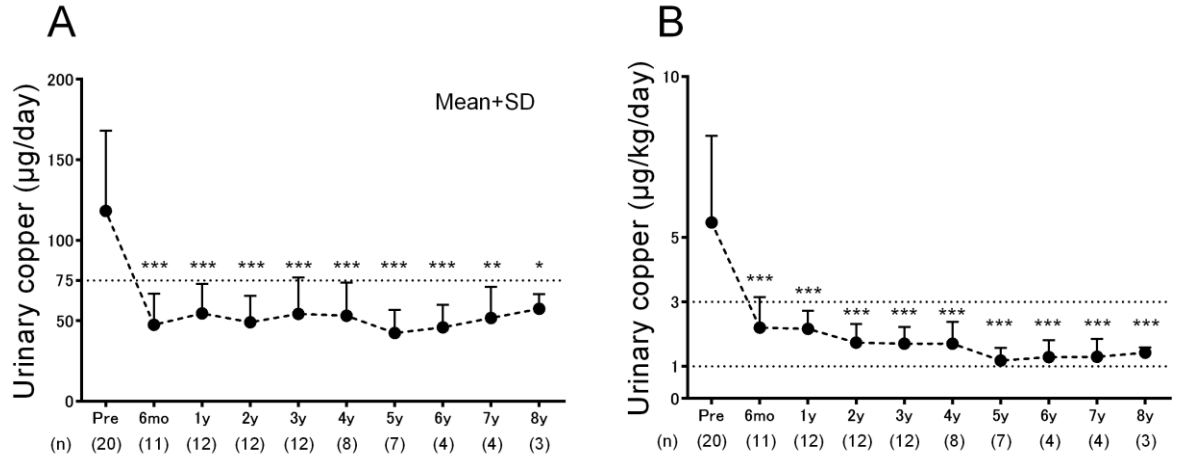
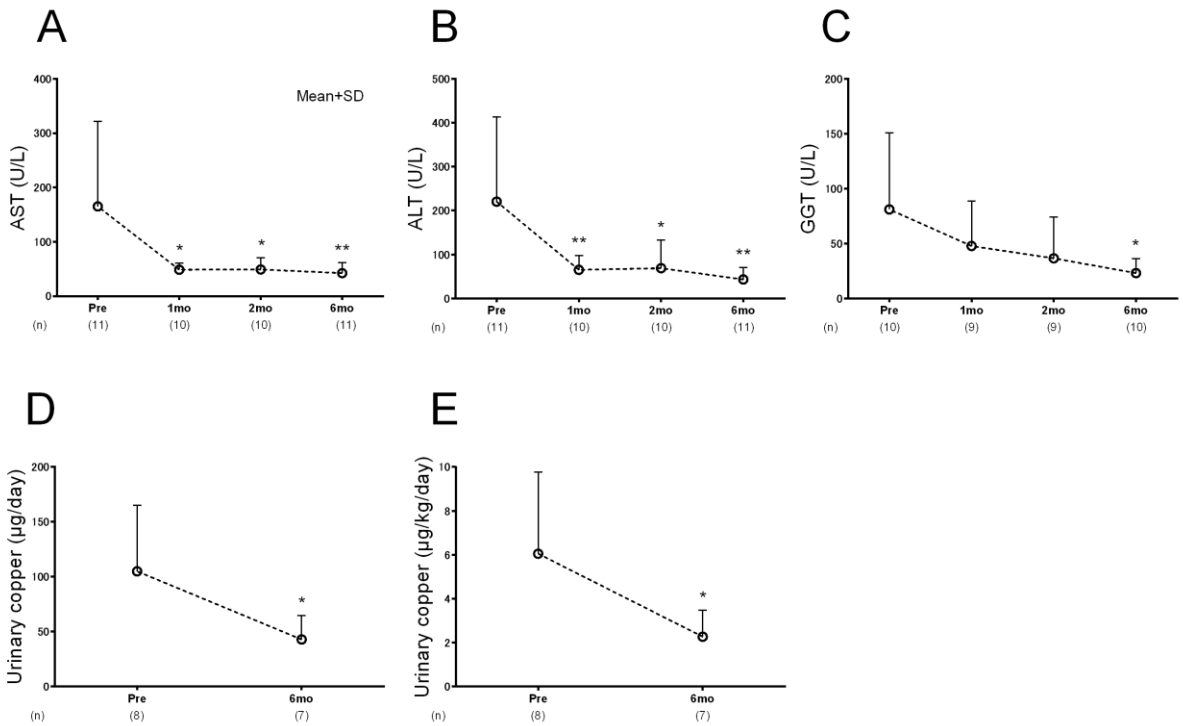
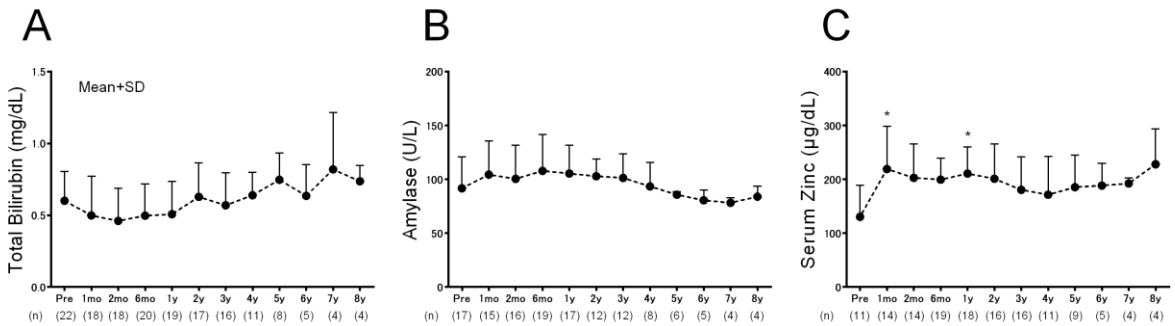


Figure 3



Supplementary Figure 1



Supplementary figure 1. Course of blood test results before and after zinc monotherapy. (A) Total bilirubin, (B) Amylase, and (C) Zinc. Pre, pretreatment; mo, month(s) after initiation of zinc therapy; y, year(s) after initiation of zinc therapy; n, number of patient at each time points; SD, standard deviation; *, $P < 0.05$ vs. Pre.